

LIMITATION OF LEFT VENTRICULAR
DYSFUNCTION AFTER ACUTE MYOCARDIAL
INFARCTION

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ABSTRACT

Myocardial infarction remains an important cause of death and disability in Scotland. Although initial mortality is largely a consequence of arrhythmias, after the first few hours mortality and morbidity is closely related to the extent of myocardial damage and subsequent ventricular dilatation. This thesis discusses means of reducing left ventricular dysfunction after myocardial infarction.

The first two sections describe a placebo controlled double blind clinical trial of oral therapy with captopril or isosorbide mononitrate initiated early after myocardial infarction and continued for 28 days. Left ventricular function and volume as assessed by echocardiography, radionuclide ventriculography or magnetic resonance imaging was similar in the placebo and vasodilator groups at 5 weeks. Even the placebo group showed a trend to a decrease in ventricular diameter. In contrast to many similar studies most of these patients (88%) received thrombolysis, perhaps resulting in a population with little tendency to ventricular dilatation.

Clinical outcome (death, cardiogenic shock) was significantly better in the captopril group, suggesting this agent may have a role for high risk patients (possibly those with persisting coronary occlusion). However there was no evidence that vasodilator therapy reduced infarct size as quantified by tomographic radionuclide imaging. In addition the acute inflammatory response, as reflected by measures of neutrophil activation and free radical lipid attack, was not modified by vasodilator therapy.

The last two sections focus on coronary artery reperfusion. A detailed

study of 17 patients with myocardial infarction found streptokinase therapy caused complement and neutrophil activation, particularly in those that did not reperfuse. This inflammatory activation may lead to a detrimental increase in infarct size. The pattern of complement activation was consistent with plasmin mediated proteolytic activation rather than an antigen-antibody reaction.

The last section describes a retrospective review of cases of ventricular septal rupture post infarction and demonstrates that thrombolysis does not modify the presentation of this complication. Rupture almost always occurred in the context of failed reperfusion. This data refutes the theory that rupture is a consequence of harmful intramyocardial haemorrhage secondary to reperfusion.

In conclusion, left ventricular dilatation post myocardial infarction is decreased by ensuring patency of the infarct related coronary artery. Thrombolysis readily achieves this in the majority of cases, but it is associated with potentially deleterious acute inflammatory activation. Thrombolysis has not modified the presentation of septal rupture; it is still seen in those that fail to reperfuse. In high risk individuals vasodilator therapy may have a role in the prevention of progressive ventricular dilatation.

FORMAL DECLARATION

I declare that I have written this dissertation presented to the University of Edinburgh for the degree of Doctor of Medicine; that it is based on my own observations and that, except as acknowledged in the thesis, the data were collected, analysed and interpreted by me. This thesis has not been submitted for any other degree.

ALLISTER D HARGREAVES

CHAPTER 1

INTRODUCTION

MYOCARDIAL INFARCTION

Myocardial infarction remains the commonest cause of death in middle aged men in Scotland. In addition the Scottish mortality rate from myocardial infarction is one of the highest national rates in the world (Uemura and Pisa, 1988). For example, in 1989 the mortality rate from myocardial infarction for men aged 55-64 years was 559.6 per 100,000 in Scotland compared to a rate of 348.3 per 100,000 in England and Wales (World Health Statistics, 1990) and only 112.6 per 100,000 in France (World Health Statistics, 1991). The size of the problem in Scotland can be appreciated when one considers that the mortality rate from lung cancer for Scottish men in the same age group is only 247.3 per 100,000 (World Health Statistics, 1990).

Over the last 15 years much has been learnt about the pathological processes that occur during and after myocardial infarction. This improvement in understanding has led to important changes in treatment and outcome of this very common condition.

PATHOLOGY

The clinical syndrome of myocardial infarction was first described by Obrastrow and Straschenko (1910). Two years later, in 1912, Herrick reported his description of the 'clinical features of sudden obstruction of the coronary arteries'. In both these papers coronary artery thrombosis was clearly stated as the cause of the clinical syndrome. In the mid 20th century there was debate as to whether coronary thrombosis was the cause or the result of myocardial infarction (Hackel et al, 1969). As recently as 1980 Silver et al wrote of the lack of importance of coronary thrombosis in myocardial infarction. In their autopsy series of 100

patients with myocardial infarction only 45% had occlusive coronary artery thrombi. However, in the same year DeWood et al (1980) reported their findings from coronary angiography which confirmed the importance of thrombotic occlusion of a coronary artery in the early hours after myocardial infarction. Less than 4 hours after the onset of pain, 97.3% of patients had a total or subtotal occlusion of the relevant coronary artery. Recently percutaneous angioscopy has allowed visualisation of such thrombi in vivo. Greyish-white platelet rich thrombi are seen in patients with unstable angina, whereas occlusive reddish thrombi predominantly comprising fibrin and erythrocytes are found in subjects with myocardial infarction (Mizuno et al, 1992).

Coronary thrombosis is initiated by rupture of an atherosclerotic plaque (Falk, 1983; Davies and Thomas, 1985). The deeper the fissure the more likely the thrombotic process will result in coronary artery occlusion (Davies and Thomas, 1985). However, it is not a prerequisite that an atherosclerotic plaque should be large and causing a severe stenosis prior to rupture. In an angiographic study two thirds of the plaques that had progressed to thrombosis had not even been haemodynamically significant (less than 60% stenosis) prior to the event. Similarly in a prospective angiographic study the majority of lesions (85%) that caused clinical myocardial infarcts were not severe (less than 75% stenosis) at their initial assessment (Webster et al, 1990).

Once the artery is occluded by the superimposed thrombus, myocardial necrosis ensues. Thankfully this does not occur instantly following cessation of blood flow or techniques like coronary angioplasty would

not be possible without causing infarction. Animal studies suggest that myocyte injury progresses from an initial reversible state to irreversibility and necrosis (Reimer and Jennings, 1979). These workers demonstrated that in the dog the wavefront of necrosis starts in the endocardium after 20 minutes of occlusion and spreads towards the epicardium as the duration of occlusion is prolonged. In this animal the degree of necrosis has reached its maximum ('transmural infarction') within 6 hours of arresting flow.

Even if an artery remains occluded permanently, myocardial necrosis does not normally involve the entire thickness of the ventricular wall. A rim of epicardium remains viable. These cells are preserved by epicardial collateral flow which in the dog varies between 1% and 41% of the preocclusion blood flow (Reimer and Jennings, 1979). The magnitude of this collateral flow is a major determinant of the extent of myocardial necrosis. Indeed this accounts for the difference in infarct size between different experimental models. In the dog, which has a well developed collateral blood supply, coronary occlusion produces a transmural infarct in only 54% of cases (Eaton et al, 1981). In contrast the same procedure in the rat results in transmural damage in 95% of animals because of the poorer collateral blood flow in this species (Hochman and Bulkley, 1982).

The greatly enhanced collateral flow seen in some patients with chronic ischaemic heart disease can result in significant prolongation of the time course of cell death such that myocardial salvage is possible beyond 6 hours (Jennings and Reimer, 1983). This protective effect of collateral blood flow is illustrated by the observation that a third of patients with

non Q wave infarction have persisting occlusion of the offending coronary artery, but with retrograde perfusion of that artery by collateral vessels (DeWood et al, 1986). Indeed it is possible for an artery to occlude without causing infarction if collateral flow is sufficiently developed (Webster et al, 1990).

Coronary thrombosis is a dynamic process with some patent vessels progressing to total occlusion and others reopening as a result of spontaneous fibrinolysis (DeWood et al, 1980; DeWood et al, 1986). Over the first 24 hours after the onset of chest pain there is a decrease in the proportion of patients with coronary artery occlusion (DeWood et al, 1980). During this time 35% of patients will spontaneously reperfuse the occluded artery (DeWood et al, 1980). Other studies have shown increasing arterial patency rates over the weeks following infarction. Angiographic patency was seen in 41% of infarct related arteries 1 week after infarction (National Heart Foundation of Australia Coronary Thrombolysis Group, 1988) and after 3 weeks the percentage patent had risen to around 60% (White et al, 1987a; O'Rourke et al, 1988). The dynamic nature of the process of coronary thrombosis is emphasised by human pathological studies. In an autopsy series of patients with unstable angina Falk et al (1985) report that coronary thrombi have a layered structure comprising material of different ages. In addition there is evidence of previous fragmentation with microemboli in the distal circulation and microinfarcts in the same distribution.

THROMBOLYTIC THERAPY

Understanding of the importance of thrombosis in precipitating coronary artery occlusion has resulted in therapy designed to remove the

thrombus. The fibrinolytic system is the body's natural mechanism for clot lysis and depends on the enzyme plasmin attacking insoluble fibrin matrix. Active plasmin can also be generated by pharmacological manipulation with agents such as streptokinase, anistreplase and tissue plasminogen activator (reviewed by Marder and Sherry, 1988).

The first reported use of streptokinase in patients with acute myocardial infarction was in a small study by Fletcher et al in 1959. Many reports followed but the individual results were inconclusive. They tended to randomise relatively few patients and used low dose regimes which continued for many hours. The data from 33 of these studies was subjected to a meta-analysis which found that fibrinolytic therapy reduced the risk of death by 22% (Yusuf et al, 1985).

The beneficial action of thrombolytic therapy was later borne out in the large multicentre trials employing high dose but short duration treatment. In the GISSI (Gruppo Italiano per lo studio della Streptochinasi nell'infarto miocardico) study (1986) 11,712 patients were randomised to either streptokinase 1.5MU over 1 hour or no thrombolytic therapy. The 21 day mortality rate was reduced by 18% in the thrombolysed patients. Similar findings were reported by the ISIS-2 (Second International Study in Infarct Survival) trial (1988) which employed the same thrombolysis protocol (25% reduction in the odds of death in the first 5 weeks). Anistreplase, anisoylated plasminogen streptokinase activator complex, was studied in 1004 patients in the AIMS (APSAC Intervention Mortality Study) trial (1988) and reduced the 30 day mortality by 47%. The mortality rate 1 month after infarction was decreased by 26% in those treated with recombinant tissue-type

plasminogen activator in the ASSET (Anglo-Scandinavian Study of Early Thrombolysis) study (Wilcox et al, 1988).

As a result of these and other studies thrombolytic therapy has become the standard treatment for acute myocardial infarction. Two of these large studies indicated that the benefit of treatment was greater when it was given earlier after the onset of pain (ISIS2, 1988; GISSI, 1986) and this has led to efforts to ensure that patients are treated as soon as possible after presentation. Angiographic studies have confirmed that thrombolytic therapy does increase the proportion of patients with patent infarct related arteries both acutely and in the longer term after infarction (White et al, 1987a; National Heart Foundation of Australia Coronary Thrombolysis Group, 1988; O'Rourke et al, 1988).

REPERFUSION INJURY

Lysis of an occluding coronary thrombus (both spontaneous and drug induced) results in reperfusion of ischaemic myocardium. Kloner et al (1974a) have described the morphological changes that occur rapidly following reperfusion in the canine myocardium. Within 10 minutes of reperfusion the cells which have suffered "irreversible injury" swell and exhibit contraction band necrosis with large granular mitochondrial densities. There has been considerable debate concerning the possibility that such reperfusion might cause myocardial damage ("reperfusion injury"). The main body of evidence that supports the existence of reperfusion injury has been derived from animal models that have demonstrated that a variety of pharmacological interventions can decrease the extent of myocyte injury caused by a period of ischaemia and reperfusion. For instance Romson et al (1983) described an

experiment using an open chest dog model of infarction that involved reperfusion after coronary occlusion for 90 minutes. Infarct size was reduced by a mean of 43% in those dogs depleted of circulating neutrophils by pretreatment with neutrophil antiserum.

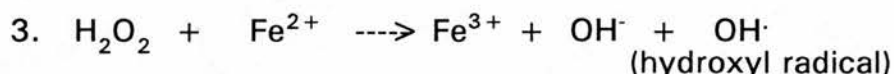
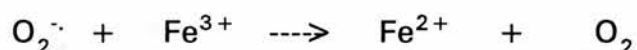
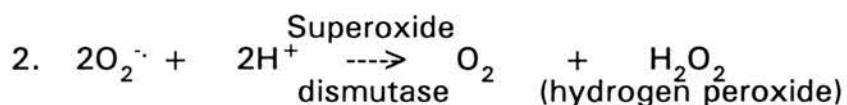
As yet there is no direct evidence from clinical studies to indicate that reperfusion injury exists in man. Indeed reperfusion induced by thrombolytic therapy lowers mortality after myocardial infarction (GISS1, 1986; ISIS-2, 1988) and if achieved early after the onset of chest pain, at least some of the benefit emanates from a decrease in infarct size (White et al, 1987a; O'Rourke et al, 1988). Although the beneficial consequences of reperfusion are clear, this does not exclude the possibility that adjuvant therapy to limit reperfusion injury might bring even greater reductions in infarct size. There are a number of different mechanisms that have been proposed as mediators of myocyte injury in the context of myocardial infarction and reperfusion. These are reviewed in the next section and the potential for adjuvant therapy to decrease such injury is discussed where relevant.

MEDIATORS OF INJURY

a) Oxygen free radicals

These molecules have an unpaired electron and are highly reactive and cytotoxic. These chemical species can modify important cellular molecules including lipids, proteins and nucleic acids. They can both cause injury and be produced as a result of injury. Oxygen can react to produce several such molecules.





This chain of reactions leads to the production of the highly reactive hydroxyl radical which is probably the most important of the oxygen radicals in causing biological effects (Pyror, 1986). Studies with isolated rabbit interventricular septa have shown that perfusion with hydroxyl radical generating media causes extensive morphological changes to both myocytes and endothelial cells, and decreases muscle function (Burton et al, 1984). Superoxide dismutase, which scavenges the superoxide radical and prevents the formation of the hydroxyl radical, protected against these changes when it was added to the perfusate.

There are several potential sources of free radicals in the context of myocardial infarction.

1) Xanthine oxidase system (McCord, 1985)

As a result of ischaemia ATP is degraded through a number of intermediates to hypoxanthine. In addition xanthine dehydrogenase is converted to xanthine oxidase by calcium dependent proteases. Hypoxanthine is then able to react with oxygen to form xanthine which becomes uric acid with the production of superoxide free radicals. The importance of this reaction in man is uncertain as there is good evidence that the

enzyme, xanthine dehydrogenase, is not present in human myocytes (Eddy et al, 1987). However it is present in the capillary endothelium so at least endothelial damage is possible as a result of this reaction (Jarasch et al, 1986).

2) Activated neutrophils (Weiss, 1989)

The NADPH oxidase system is a membrane associated enzyme complex that can generate oxygen centered free radicals when the neutrophil becomes 'activated'. Electrons shuttle from the cytosolic NADPH to oxygen molecules to produce superoxide radicals. As a result of dismutation two superoxide radicals form one molecule of hydrogen peroxide. At present it is uncertain if the hydroxyl radical is produced by the activated neutrophil generating hydrogen peroxide (Britigan et al, 1988).

It is perhaps unlikely that neutrophils contribute to the very early (first 5 minutes) burst of free radical activity that may be seen after reperfusion in a dog model of infarction (Bolli et al, 1988). Animal work indicates that neutrophil margination within the reperfused vascular bed does not occur until at least 20 minutes after the vessel has reopened (Sommers and Jennings, 1964) and that neutrophil infiltration only follows a coronary occlusion lasting more than 12 minutes (Go et al, 1988).

3) Mitochondrial electron transport

It has been proposed that ischaemia results in increased leakage of electrons from the mitochondrial electron transport

chain and these might generate free radicals (McCord, 1988). Ischaemia could cause this by a decrease in the adenine nucleotide pool and a loss of the myocardial defences against oxidative damage (Ferrari et al, 1985).

4) Arachidonic acid metabolism

There is evidence that prostaglandin H synthetase and lipoxygenase can generate superoxide radicals at least in vitro (Rowe et al, 1983; Kukreja et al, 1986).

5) Oxidation of catecholamines

It seems unlikely that 'autooxidation' of catecholamines is an important source of free radicals as this process is very slow at physiological pH (Jewett et al, 1989). However oxygen centred free radicals may be produced as a result of catalysed oxidations involving enzymatic systems and/or metal ions.

It is difficult to study free radicals in vivo because they are highly reactive and are only present in very small concentrations at any given time. In vitro techniques such as pulsed radiolysis are used to measure the reaction constants for different chemical reactions involving free radicals (Halliwell and Gutteridge, 1989). Briefly radicals are formed within a reaction cell by a pulse of ionising radiation and the appearance and disappearance of radical species can be detected by changes in the absorbance characteristics over the following microsecond. By changing the conditions and the chemicals within the chamber different radical species can be studied.

Free radicals can be measured in biological systems using electron spin resonance spectroscopy. The unpaired electron in a free radical behaves as a very small magnet and will orient either with or against an applied magnetic field. This results in the electron having one of two energy states which can be detected by measuring the absorbance spectrum from an external source of electromagnetic radiation. Electron spin resonance is very sensitive but still requires the radical to remain long enough to be measured. This problem can be circumvented by the use of a "spin trap", a chemical which combines with highly reactive radicals and produces a long lived more stable radical that can be more readily measured. α -Phenyl-tert-butyl nitron (PBN) and 5,5-dimethylpyrroline-N-oxide (DMPO) are examples of two such spin traps. These chemicals have been used to detect free radical production in experimental animals but only in a limited fashion in man (Coghlan et al, 1991). For example Bolli et al (1988) used electron spin resonance spectroscopy with the spin trap, PBN, to detect free radical species in their canine model of myocardial stunning.

An alternative approach that might be applicable to work in vivo is the administration of an aromatic compound such as salicylic acid. The hydroxyl radical reacts with salicylic acid to produce 2,3- and 2,5-dihydroxybenzoates. The 2,3 isomer is not thought to be produced by other enzymatic pathways and therefore the concentration should represent hydroxyl radical formation (Grootveld and Halliwell, 1986). Other workers have suggested that the 2,5 isomer of dihydroxybenzoate more closely reflects free radical activity (Udassin et al, 1991). Although this method shows great promise it remains to be fully evaluated.

Free radical attack on lipids results in lipid peroxidation which can be

quantified by a number of different methods, providing an indirect measure of free radical activity. Diene conjugates (unsaturated fatty acids with two double bonds only separated by one single bond) are formed early in the process of peroxidation after the abstraction of a hydrogen atom from an unsaturated fatty acid. This isomeric structure is favoured as it gives the carbon centred radical more stability. Conjugated dienes are detected by measuring UV absorbance at 230-235 nm. Dormandy and his colleagues have refined this method by including separation of biological material by high performance liquid chromatography and also by the employment of the ratio of native linoleic acid (18:2(9,12)) to its diene conjugate (18:2(9,11)) (Iversen et al, 1985). This technique has been criticised by Thompson and Smith (1985). They state that the diene conjugate of linoleic acid is unlikely to be formed *in vivo* as the linoleic acid radical would preferentially react with oxygen to produce a peroxy radical. Instead these authors suggest that diene conjugates may arise from the diet or the gut flora. However the oxygen tension *in vivo* is not always high, for example in the myocardium of a patient after sudden coronary occlusion. Conjugated diene levels are elevated in other disease states and are thought to correlate with free radical activity e.g. alcoholic liver disease (Hayes et al, 1989), preeclampsia (Erskine et al, 1985) and rheumatoid arthritis (Lunec et al, 1981).

Lipid peroxidation can result in the formation of hydrocarbon gases and these can be measured in exhaled air (Tappel and Dillard, 1981). This process is favoured at low oxygen tensions but is complicated by liver metabolism of the hydrocarbon gas and external pollution. For these reasons the data from this technique is prone to error.

One of the oldest and most frequently employed assays of lipid peroxidation is the thiobarbituric acid test which involves heating biological material with thiobarbituric acid under acid conditions and detecting the formation of a pink colour by measuring absorbance at 532 nm. The basic reaction is between malondialdehyde, one of the many end products of peroxidation, and thiobarbituric acid to produce a coloured product. Unfortunately malondialdehyde is very reactive and the plasma concentration is very low. Much of the measured malondialdehyde is produced during heating from lipid peroxides present in the test tube. The test has poor specificity as many other chemicals (various sugars, biliverdin, sialic acid and haemolysed serum) react with thiobarbituric acid to give a coloured product (Knight et al, 1988). Some workers have used high performance liquid chromatography to separate the malondialdehyde-thiobarbituric acid adduct and so increase specificity (Wong et al, 1987). Unfortunately this still does not distinguish between malondialdehyde produced by free radical lipid peroxidation and that from endoperoxides produced by the prostaglandin synthesis pathway.

Rather than measuring free radicals or the products of free radical reactions it is also possible to measure changes in biologically active scavenging molecules (antioxidants). The main enzymatic antioxidants are catalase, superoxide dismutase and glutathione peroxidase. β -Carotene, ascorbate, vitamin A, vitamin E and sulphhydryl containing compounds are the most important non-enzymatic scavengers of free radicals. McMurray et al (1990) have measured the signal from reduced glutathione, an intracellular free radical scavenger, in red cells by proton nuclear magnetic resonance spectroscopy. Glutathione peroxidase, superoxidase and vitamin E levels were assessed in a study by Beard et al (1992). These

indices are indirect and changes in their concentration/activity are not necessarily related to free radical activity, but they are useful as confirmatory tests.

Free radical involvement in reversible myocardial dysfunction after ischaemia (myocardial stunning) has been well studied in animals. In a dog model, reperfusion after 15 minutes of ischaemia resulted in free radical production which was detected using a spin trap and electron paramagnetic resonance spectroscopy (Bolli et al, 1988). In a different animal model free radical activity associated with reperfusion increased in parallel with the duration of the preceding ischaemia (up to 90 minutes) (Arroyo et al, 1987). In other laboratory experiments dramatic reductions in myocardial dysfunction after reperfusion were seen using a number of agents that protect against free radical mediated damage (Myers et al, 1986; Bolli et al, 1987; Bolli et al, 1989). This effect was only seen when these agents were administered prior to or at the time of reperfusion (Bolli et al, 1989). A delay in treatment, by as little as one minute after the restitution of flow, resulted in loss of effect.

A considerable number of laboratory studies have assessed the antioxidant effect of superoxide dismutase on myocardial infarct size. Initial reports indicated that superoxide dismutase therapy, started before reperfusion, significantly decreased infarct size (Jolly et al, 1984; Werns et al, 1985; Ambrosio et al, 1986). However more recently there have been many other reports that failed to demonstrate any beneficial effect (Uraizee et al, 1987; Richard et al, 1988; Nejima et al, 1989).

It has been suggested that the half life of superoxide dismutase in vivo is

too short and that benefit would result from modification of the enzyme to prolong its half life. Unfortunately the results of studies using modified superoxide dismutase are still conflicting. Tamura et al (1988) and Chi et al (1989) both found evidence of myocardial salvage whereas Ooiwa et al (1989) and Tanaka et al (1989) did not find such activity.

The discrepancy between these results is now explicable since Przyklenk and Kloner (1987a) demonstrated that superoxide dismutase only delays myocyte necrosis when assessed by tetrazolium staining. If the assessment of myocyte necrosis is histological and therefore by necessity at least 24 hours after reperfusion then superoxide dismutase does not reduce infarct size. This methodological artefact has been clarified by a Japanese group who showed that 24 hours after reperfusion tetrazolium staining indicated infarct size to be only 50% of the histological extent of myocyte necrosis in animals treated with superoxide dismutase (Shirato et al, 1989).

The lack of convincing benefit from treatment with superoxide dismutase does not mean that free radicals are not important in causing myocyte damage. It may be that a different scavenging agent is required. N-2-mercaptopropionyl glycine is an antioxidant which directly scavenges hydroxyl radicals by virtue of its sulphhydryl group and may reduce infarct size. Mitsos et al (1986) studied this agent in a dog model of infarction which included prior neutrophil depletion. Infarct size was decreased by 33% by reducing the number of circulating neutrophils and was decreased further (by 63%) in those dogs that were treated by both neutrophil depletion and N-2-mercaptopropionyl glycine (MPG). Apart from indicating a role for a free radical scavenger in reducing infarct size,

this study suggests that free radical production during and after reperfusion is not purely from activated neutrophils. The ability of MPG to cross cell membranes may be important in permitting access to the site of radical production and therefore explain why this agent is effective in suppressing free radical damage (Lesnefsky, 1992).

There is evidence of free radical activity following myocardial infarction in man, but its relationship to reperfusion is not clear. Clinical investigation has demonstrated that conjugated diene levels are increased after infarction. Bell et al (1990) found that neither the extent nor the timing of this elevation was influenced by thrombolytic therapy. Grech et al (1992) have also reported elevated levels of conjugated dienes in patients with acute myocardial infarction but in their study this elevation occurred within 10 minutes of successful primary angioplasty. The source of the free radical marker appeared to be the myocardium as the blood samples with the highest levels were drawn from a catheter in the coronary sinus. Grech et al found that malondialdehyde levels were not increased either before or in the 48 hours after angioplasty. A different group has reported that free radical activity may be associated with successful thrombolysis as they found an increase in malondialdehyde production 2 hours after thrombolytic therapy in those with angiographic evidence of reperfusion (Davies et al, 1990a). In contrast to the other reports conjugated diene production was not increased in these patients. Using an alternative marker of free radical activity Beard et al (1992) found that vitamin E concentrations decreased over the first 48 hours after thrombolytic therapy in patients with acute infarction. Workers from the USA have shown that patients with myocardial infarction have significantly higher breath pentane levels than patients admitted with chest pain without

infarction (Weitz et al, 1991). This study applied their measure of free radical activity on admission prior to any attempt at therapeutic reperfusion.

Further work is required to elucidate the relationship between free radicals and reperfusion in man. The source of the free radical activity after infarction remains unknown and it is still not clear if this activity is clinically harmful by increasing the extent of myocyte necrosis in man. Free radical scavenging agents that are beneficial in animal studies and are non toxic will naturally require careful testing in man. The most promising drugs are the membrane soluble sulphydryl containing compounds.

b) Calcium overload

One of the consequences of reperfusion observed in animal experiments is a rapid and excessive influx of calcium ions into myocardial cells. This is not seen in non reperfused myocardium and neither does it occur in reversible ischaemic myocardial dysfunction following shorter periods of coronary occlusion (Shen and Jennings, 1972a). Much of the calcium appears as precipitates in mitochondria (Shen and Jennings, 1972b). Greater calcium uptake is seen after longer periods of ischaemia and as one would expect this correlates with a poorer recovery of myocardial function (Bourdillon and Poole-Wilson, 1981). Calcium influx occurs at a time when the cell membrane is not disrupted and the process can be inhibited by nickel or cyanide ions (Poole-Wilson et al, 1984). However it seems most likely that calcium influx occurs in myocytes that have already suffered irreversible damage and it is not a primary cause of cell destruction.

c) Oedema

Reperfusion causes rapid and significant cellular oedema which is most apparent in myocytes rather than the capillary endothelium (Kloner et al, 1974a). Within 2 minutes of reperfusion the myocardial water content has risen by 20% (Whalen et al, 1974). This phenomenon is not seen in non reperfused myocardium. By virtue of external capillary compression the increase in intracellular water may be responsible for the observed increase in coronary vascular resistance that follows reperfusion (Powers et al, 1984), and may increase the ischaemic insult.

d) Intramyocardial haemorrhage

Permanent coronary artery occlusion results in an area of infarction that is pale and not haemorrhagic. In animal studies between 44 and 100% of infarcts are haemorrhagic following experimental mechanical reperfusion (Bresnahan et al, 1974; Capone and Most, 1978; Roberts et al, 1983). In the anaesthetised dog it is clear that haemorrhage only occurs in the context of severe ischaemia in tissue that is already irreversibly injured (Higginson et al, 1982). The extent of haemorrhage is greatest in the endocardium and also in animals with the lowest collateral blood flow. In addition in later experiments Higginson et al (1983) reported that the extent of haemorrhage was greater when the duration of experimental occlusion was longer. On histological examination haemorrhage is confined to areas of necrosis (Higginson et al, 1982). Concern has been raised that haemorrhage may increase infarct size as reported by Bresnahan et al (1974). However this conclusion may be invalid as in this animal study the extent of necrosis was only assessed by creatine kinase curves and not by histology. It is reassuring that Roberts et al (1983) reported haemorrhage to be associated with a lesser neutrophil infiltrate

and no impairment of scar healing. Thus it seems likely that haemorrhage is secondary to established necrosis and probably follows ischaemic injury to the capillary endothelium. It is interesting that an animal study has shown that streptokinase does not increase the extent of haemorrhage beyond that seen after non thrombolytic reperfusion, despite the dramatic effects that this drug has on the clotting cascade (Kloner and Alker, 1984).

In post mortem series the majority of patients who received streptokinase had a grossly haemorrhagic infarct (Mathey et al, 1982; Waller et al, 1987). However, as these studies were limited to those who died after thrombolysis, they may not be representative of the survivors who received such therapy. In contrast to animal studies haemorrhage does not appear to occur following mechanical reperfusion by primary coronary angioplasty in man (Waller et al, 1987). There is continuing debate as to the potential harm that might ensue from intramyocardial haemorrhage, particularly after therapeutic thrombolysis. Delayed treatment with thrombolytic agents may increase the incidence of cardiac rupture post infarction (Mauri et al, 1987; Honan et al, 1991). Honan et al postulated that dissection of blood between myocyte bundles might lead to tissue disruption and cardiac rupture.

d) No reflow phenomenon

In 1966 Krug et al reported that temporary occlusion of a canine coronary artery followed by reperfusion resulted in an area of absent perfusion in the endocardium despite a widely patent epicardial vessel. The term 'no reflow' phenomenon was applied several years later (Kloner et al, 1974b). This latter group documented that this phenomenon was only apparent if

the period of coronary artery occlusion lasted longer than 40 minutes. They confirmed that it occurred predominantly in the endocardium and they described the histological findings of extensive capillary damage and myocardial cell swelling (contraction band necrosis). A later and more detailed study of the time course of the ultrastructural changes following ischaemia showed that microvascular damage lags behind myocyte necrosis indicating that it is unlikely that the microvascular changes cause myocyte injury (Kloner et al, 1980).

It has been suggested that 'no reflow' may be a result of myocardial or endothelial cell swelling mechanically obstructing capillary flow. Alternatively reperfusion studies indicate that microvascular plugging may be caused by platelets (Feinberg et al, 1982) and neutrophils (Engler et al, 1983; Engler et al, 1986). Fibrin plugs per se are probably not responsible for this lack of perfusion as it is still seen after streptokinase therapy (Kloner and Alker, 1984).

It has become clear that the 'no reflow' phenomenon is more complex than was initially thought. The extent of impairment of myocardial perfusion gradually increases over the first few hours after reperfusion (Ambrosio et al, 1989; Jeremy et al, 1990). The area of immediate no reflow is characterised by severe coagulative necrosis on histological examination and an absence of collateral flow during the ischaemic period (Ambrosio et al, 1989). In contrast in areas of delayed impairment of flow, histology reveals both contraction band necrosis, as has been described in 'reperfused' myocardium, and a significant intravascular accumulation of neutrophils (Ambrosio et al, 1989).

It is not surprising that neutrophil depletion in animal studies decreases the impairment of perfusion which follows restoration of coronary flow (Chatelain et al, 1987; Litt et al, 1989). In one report there was also a decrease in infarct size in the neutrophil depleted group (Litt et al, 1989). However persisting benefit from inhibition of neutrophil function was only seen if this state was maintained for 48 hours after reperfusion, as demonstrated in a study using a prostacyclin analogue infusion (Simpson et al, 1988a). It is interesting that after reperfusion there is even depressed blood flow in the reperfused epicardium that does not exhibit necrosis. The importance of neutrophils in the 'no reflow' phenomenon has not been confirmed in all studies. Neutrophil depletion failed to prevent the impairment of perfusion that followed 2 hours of ischaemia in the dog (Carlson et al, 1989).

If the 'no reflow' phenomenon exists and is harmful in man, then there would be potential for therapeutic manipulation to improve myocardial perfusion and perhaps salvage tissue. Evidence for this is still awaited but the most likely target for such therapy is the acute inflammatory response.

f) Neutrophil activation

There is little doubt that neutrophils are involved in the process of myocardial infarction. Early pathological studies demonstrated neutrophil invasion within 24 hours of the onset of chest pain (Mallory et al, 1939; Fishbein et al, 1978). Clinical studies have shown evidence of their activation in the hours and days after infarction. Mehta et al (1989) drew peripheral blood from patients within 1 hour of the onset of chest pain and found altered neutrophil morphology and elevated levels of peptide B β (a product of fibrin degradation by the neutrophil enzyme, elastase)

confirming neutrophil activation. Similar activation was documented 3 days after infarction by Nash et al (1989) using morphological assessment and measurement of white cell filterability. In our own laboratory, Bell et al (1990) found increased plasma levels of neutrophil elastase which persisted for up to 48 hours after infarction. Using nuclear imaging they demonstrated myocardial uptake of autologous indium-111 labelled neutrophils provided they were reinjected within 24 hours of the onset of chest pain (Bell et al, 1987). Those patients who had received thrombolysis had higher levels of plasma elastase early after infarction although nuclear imaging revealed a relative decrease in myocardial neutrophil uptake (Bell et al, 1990). In contradiction, Ranjadayalan et al (1991a) found neutrophil elastase levels to be elevated 1 hour after streptokinase infusion but to revert to normal within 6 hours. No attempt was made to elucidate the time course of neutrophil activation over the initial 2 hour period. In a more comprehensive report Ranjadayalan et al (1991b) state that neutrophil activation was more marked in those with reperfusion, but unfortunately this was only assessed 5 days later by angiography. In addition they found a negative correlation between elastase measurements and left ventricular ejection fraction. They suggested that this was evidence of possible myocardial injury by neutrophils. It is perhaps more likely that the neutrophil response is simply dependent on the extent of infarction. Part of the discrepancy between Ranjadayalan et al (1991a) and Bell et al (1991) may be accounted for by the choice of different assays for elastase. The former used a commercial kit that employed a double antibody sandwich enzyme linked immunoadsorbent assay to elastase complexed to $\alpha 1$ proteinase inhibitor whereas the latter used a specific radioimmunoassay with rabbit polyclonal antiserum to both free and complexed neutrophil elastase. In a

more recent report Davies et al (1992) found increasing elastase concentrations over the first 48 hours after the onset of pain and also a significant correlation between the levels of elastase and thiobarbituric acid reactive material, suggesting that neutrophils are a source of free radicals later after myocardial infarction. Thus neutrophil activation occurs after infarction, but the interaction between the human neutrophil and thrombolysis mediated reperfusion remains to be fully elucidated.

Laboratory investigation suggests the activated neutrophil may be involved in reperfusion injury which may result from other mechanisms than simple mechanical capillary occlusion (discussed above). Activated neutrophils produce oxidants that can mediate damage. The neutrophil NADPH oxidase system produces superoxide radicals and hydrogen peroxide. In addition neutrophil granules contain the enzyme myeloperoxidase that is capable of catalysing the reaction of hydrogen peroxide and chloride ions to produce the reactive oxidant, hypochlorous acid (Harrison and Schultz, 1976). Activated neutrophils can generate large quantities of this acid by this means (Weiss et al, 1982). Although hypochlorous acid has little systemic toxicity when used to irrigate surgical wounds, it is toxic when in direct contact with the delicate vascular endothelium (Dakin, 1916; Johnson et al, 1987).

Another mechanism of neutrophil mediated injury involves the neutrophil granule enzymes, of which the most important are the proteases: elastase, collagenase and gelatinase (Weiss, 1989). Elastase has widespread destructive properties, being able to degrade both extracellular matrix and plasma proteins, and can even damage intact cells (Janoff, 1985). The body defends itself against such proteinases by possessing powerful

antiproteinases such as α_1 -antiproteinase inhibitor, α_2 -macroglobulin and secretory leucoproteinase inhibitor (Janoff, 1985). In a fascinating example of synergism the neutrophil uses its oxidant mechanisms to facilitate damage caused by elastase and the other proteinases. Neutrophil derived oxidants inactivate all three antiproteinases (Weiss et al, 1984; Weiss et al, 1986; Kramps et al, 1987) and in addition they activate the latent collagenase and gelatinase enzymes that are secreted from neutrophil granules (Weiss et al, 1985; Peppin and Weiss, 1986).

Unfortunately the neutrophil's destructive powers are not selective once stimulated and therefore this cell has the potential to damage viable cells that lie adjacent to necrotic or injured cells. It is in this capacity that neutrophils have been investigated as mediators of reperfusion injury. Intervention experiments with animal models of coronary artery occlusion and reperfusion have revealed promising results. Neutrophil depletion by antibodies (Romson et al, 1983; Jolly et al, 1986) or leucocyte filters (Litt et al, 1989) resulted in significant decreases in infarct size. Similar effects have been obtained using antibody directed against leucocyte adhesion molecules (anti-Mo1, anti-CD11b) (Simpson et al, 1988b; Simpson et al 1988c). Both prostacyclin (PGI_2) and prostaglandin E_1 (PGE_1) inhibit neutrophil activation and have been shown to decrease infarct size in animal models (Simpson et al, 1987a; Simpson et al, 1987b; Simpson et al, 1988a; Simpson et al, 1988d).

The efficacy of these interventions depends on the duration of ischaemia being relatively short (around 90 minutes). When coronary occlusion is prolonged for 3 hours or longer no benefit is seen from reducing neutrophil activity (Jolly et al, 1986; Chatelain et al, 1987). This may be because

after this time the wave of coagulative necrosis has extended to include a large part of the thickness of the myocardium and there is little possibility of salvage of reversibly injured myocytes. Studies of neutrophil mediated reperfusion injury have also shown a requirement for neutrophil function to be suppressed for 48 hours after reperfusion if a long term beneficial effect is to be seen (Simpson et al, 1988a).

g) Complement

The complement system in man comprises 20 proteins which have a key role in orchestrating the acute inflammatory response (Muller-Eberhard, 1988). Activation normally occurs either through the classical pathway by antigen antibody complexes or via the alternative pathway by a heterogenous group of particles which include polysaccharides, fungi, viruses, bacteria and some mammalian cells. Such complement activation results in amplification of the acute inflammatory response (Goldstein, 1988).

Anaphylotoxins (C3a, C4a and C5a) are produced by the complement pathway and cause increased capillary permeability, smooth muscle contraction, histamine release and neutrophil activation (chemotaxis, degranulation, increased oxidative metabolism, surface adherence and expression of phagocytic (C3) receptors). Complement proteins are also surface bound. In particular C3b and C3bi are potent opsonins that cause phagocyte adherence and subsequent ingestion of coated particles or cells. In addition to amplifying the acute inflammatory response the complement proteins are capable of inflicting direct damage to cells through construction of the membrane attack complex (C5b-9). This hydrophobic molecular complex inserts into cell membranes and forms a

transmembrane channel that permits bidirectional ionic flow. If sufficient numbers of these complexes are deposited on a cell membrane then cell lysis results.

Animal studies confirm complement activation in relation to infarcted tissue. In a baboon model of infarction without reperfusion there is evidence of C3 deposition on myocardial cells within 4 hours of coronary artery occlusion (Pinckard et al, 1980). After 24 hours there is extensive C3 accumulation on necrotic or abnormal myocytes (Pinckard et al, 1980; McManus et al, 1983; Crawford et al, 1988). In a rat experiment involving coronary reperfusion after a period of ischaemia, investigators found deposition of the membrane attack complex (C5b-9) along the capillary endothelium as early as three hours after reperfusion (Weisman et al, 1990). This complement activity in association with the capillary endothelium obviously has the potential to provoke a local intravascular inflammatory response with capillary occlusion and augmented myocardial damage. Electron microscopy on infarcted tissue from one of the animal studies demonstrated complement protein associated with intracellular structures: the contractile elements, nuclear membrane, mitochondrial membranes and sarcoplasmic reticulum (Pinckard et al, 1980).

There is no doubt that the complement pathways are activated in acute myocardial infarction in man. In peripheral blood samples complement activation is evident within 16 hours of the onset of chest pain (Earis et al, 1985; Langlois and Gawryl, 1988; Yasuda et al, 1989; Yasuda et al, 1990). In a human autopsy study there were large quantities of the C5b-9 complexes in areas of infarction (Schafer et al, 1986). However, evidence of complement activation was not found outside the infarcted zone and

C4 deposition was not detected.

The mode of activation of the complement pathways by ischaemic or necrotic tissue remains uncertain, but it is likely to occur by more than one route. It is clear that the myocyte or endothelial cell must change in response to ischaemia to enable complement activation to occur. Normal human cells do not activate complement as they possess protective inhibitory cell surface proteins (Muller-Eberhard, 1988; Goldstein, 1988). It is not known if complement activation can be induced by a reversible ischaemic insult, or whether a cell must suffer irreversible damage before activation can occur.

Laboratory studies, both in vitro and in vivo, have shown that C1q is capable of binding to proteins in mitochondrial membranes to cause classical pathway activation (Pinckard et al, 1973; Pinckard et al, 1975; Giclas et al, 1979; Rossen et al, 1988). There is also evidence that mitochondrial membranes can activate the alternative pathway as C3 consumption continued even when the classical pathway was blocked (Pinckard et al, 1975; Giclas et al, 1979). Similarly, Rubin et al (1990) reported alternative pathway activation in a different experimental model. Reperfusion of skeletal muscle after 5 hours of ischaemia was associated with decreasing plasma levels of factor B.

Complement activation can proceed independent of the two recognised pathways through direct action of proteases (eg trypsin or plasmin) on the complement factors (Ratnoff and Naff, 1967; Taylor and Ward, 1967; Hill and Ward, 1969). Within the area of an infarct proteases may be released or generated locally and hence lead to the production of chemotactic

fragments or full complement activation.

Depletion of complement activity has been reported to decrease infarct size in animal studies and consequently the complement pathways have been implicated as mediators of myocyte injury after ischaemia. Cobra venom factor by binding to factor B to form a very powerful and stable C3 convertase can deplete all the components of the alternative pathway including C3 and factors C5 to C9. Loss of these factors also paralyses classical pathway activation. In non reperfused animal models of infarction treatment with cobra venom factor reduces neutrophil infiltration and ultimate infarct size (Maroko et al, 1978; Maclean et al, 1978; Pinckard et al, 1980; Crawford et al, 1988). Other studies have emphasised the lack of neutrophil infiltration after infarction in cobra venom treated animals and in addition there is a lack of detectable chemotactic activity in either the coronary sinus effluent or the myocardium of such animals (Hill and Ward, 1975; Hartmann et al, 1977). There is no evidence that depleting complement activity impairs infarct healing and scar formation (Maclean et al, 1978).

Rather than depleting complement activity by activation an alternative approach is to use a soluble inhibitory protein. Investigators have studied the effects of soluble CR1 (sCR1) which binds C3b and C4 and also promotes their inactivation by factor I. In the only study of complement inhibition treatment with sCR1 reduced infarct size by 44% in a reperfused rat model of myocardial ischaemic injury (Weisman et al, 1990). Complement inhibition, like depletion, did not impair infarct healing.

Thus there is good reason to identify the complement pathway as a mediator of reperfusion injury both in its own right and through its role in the production of chemoattractants for neutrophils. Although complement activation has been demonstrated in man following myocardial infarction, it is not clear if this is initiated by reperfusion. Further thrombolytic agents may interact directly with the complement pathway. Bennet et al (1987) have already shown that recombinant tissue plasminogen activator induces marked and immediate complement activation. This may be a result of the direct action of plasmin on the complement proteins. The effect of streptokinase has not been investigated in vivo but there is in vitro evidence that it can cause complement activation (Ratnoff and Naff, 1967; Taylor and Ward, 1967).

FROM MYOCYTE NECROSIS TO SCAR FORMATION

If coronary occlusion prevents myocardial perfusion for longer than 20 minutes then necrosis will follow and the extent of necrosis increases with the duration of preceding ischaemia (Reimer and Jennings, 1979). The histological changes that follow myocyte necrosis have been reported from autopsy studies (Mallory et al, 1939; Fishbein et al, 1978). Neutrophil infiltration starts within 24 hours of coronary occlusion, but does not reach its peak until 72 hours. Fibroblast proliferation, although not evident until the fourth day after infarction, has become abundant by the seventh day when there is also evidence of collagen deposition. Concomitant with these processes macrophages remove the necrotic myocytes by phagocytosis.

The progression from inflammation to healing and scar formation starts at the periphery of the infarct and eventually reaches the centre (at least in

patients not treated with thrombolytic agents). It may take as long as 5 to 6 weeks before all the necrotic tissue is removed. The fibrous scar becomes increasingly dense over the next 2 to 3 weeks until it reaches a maximum around 2 months after infarction. In vitro studies into the physical properties of the scar tissue in left ventricular aneurysms confirm that as the infarcted area heals to form a fibrous scar, it develops a greater ability to resist stretch (Parmley et al, 1973).

During this healing process the heart is exposed to the haemodynamic forces which tend to deform the ventricle. The net result is that a proportion of patients undergo changes in ventricular size and shape following infarction. This has been termed ventricular remodelling.

VENTRICULAR REMODELLING

Within a few minutes of coronary artery occlusion there is segmental cessation of myocardial contraction and systolic bulging (Picard et al, 1991). These early changes are potentially fully reversible if flow is restored prior to myocyte necrosis. Echocardiography shows that in man ventricular dilatation may be evident within 24 hours of the onset of chest pain following an acute myocardial infarct (Niemenen and Heikkila, 1976). In a detailed study Eaton et al (1979) demonstrated that in a proportion of patients (35%), there was progressive dilatation of the left ventricle over the first 2 weeks. Longer term studies indicate that in some patients this dilating process may continue for up to 6 months after infarction (Erlebacher et al, 1982; Gadsboll et al, 1989; Jeremy et al, 1989).

The early phase (first 3 days) of ventricular dilatation is entirely due to expansion of the infarcted segment (Erlebacher et al, 1984), whereas the

later phase involves expansion of both the non infarcted and the infarcted portions (Gadsboll et al, 1989). As the non infarcted segment expands it may not undergo thinning. Instead the wall thickness may be maintained in a similar fashion to volume overload hypertrophy (McKay et al, 1986). However in larger infarcts there is thinning of the non infarcted segment in addition to the infarcted segment as the ventricle is unable to compensate (Olivetti et al, 1990).

Detailed histological analysis shows that in the infarcted zone dilatation results from stretching of myocyte fibres, loss of intercellular space and most importantly, side to side slippage of myocytes (Weisman et al, 1988). The increase in circumference of the non infarcted segment occurs almost entirely from side to side slippage of myocyte bundles (Weisman et al, 1988; Olivetti et al, 1990). These morphological changes do result in haemodynamic improvement with lower filling pressures and a greater cardiac output but at the expense of greater ventricular volumes (McKay et al, 1986).

The mechanical forces that drive these changes in shape are the preload (the diastolic distension of the ventricle) and the afterload (the resistance against which the ventricle must contract in systole). The normal heart can easily cope with the resulting wall stresses. In 1957 Burton wrote emphasising that cardiac shape determines the level of wall stress in the ventricle. After a myocardial infarct there is an increase in ventricular cavity size and a decrease in wall thickness. As the law of Laplace would predict these morphological changes increase wall stress. In a rat experiment there was a greater than 7 fold increase in wall stress as a result of a large infarct and this increase was evident in both the infarcted

and the non infarcted zones (Olivetti et al, 1990).

The augmentation in wall stress is particularly marked in diastole and leads to progressive ventricular dilatation as seen in ventricular hypertrophy resulting from volume overload (Grossman et al, 1975). In this way an enlarged ventricle is exposed to increased wall stresses and this in turn provides the stimulus for further dilatation (Pfeffer and Pfeffer, 1987). High wall stress also accounts for the propensity of apical infarcts to dilate with aneurysm formation as the normal apex is the thinnest portion of the ventricle and has the highest normal wall stress (Role et al, 1978).

Left ventricular remodelling does not always result in progressive dilatation. Indeed there are observational studies documenting improvements in ventricular size and function over the months and years after myocardial infarction. Gaudron et al (1990) found that ventricular volume decreased in small myocardial infarcts between 4 days and 4 weeks after infarction. A dramatic change was even recorded in a selected group of patients with anterior myocardial infarction in whom the mean ejection fraction increased from 26% to 41% over three years (Silberberg et al, 1989). Although this measurement is only partly related to ventricular volume, this result does imply a decrease in either end-systolic or end-diastolic volume.

Ventricular dilatation is an adverse process following a myocardial infarct. Human studies have shown that it is associated with a greater incidence of cardiac failure (Erlebacher et al, 1982; Meizlish et al, 1984) and a greater mortality (Eaton et al, 1979; Meizlish et al, 1984). The detrimental effects of impaired ventricular function have been confirmed in a large

prospective study of patients with coronary heart disease. The left ventricular ejection fraction (i.e. stroke volume/end diastolic volume) proved to be the most important predictor of survival, being more important than the extent of coronary disease (Hammermeister et al, 1979). White et al (1987b) demonstrated the importance of ventricular volumes, with end systolic volume being an even greater predictor than ejection fraction for survival following acute myocardial infarction. Those with an end systolic volume greater than 130 mls had a poor outcome with only 52% surviving 5 years. The cause of death was 'sudden' in 70%, reflecting the increased incidence of malignant ventricular arrhythmias in these patients. Only 8% died of cardiac failure. Patients with ventricular remodelling and dilatation are also at risk of mural thrombus formation with a proportion being complicated by systemic embolism (Asinger et al, 1981; Keren et al, 1990). It has been suggested that if the dilating process is rapid in the days after infarction, then cardiac rupture either of the free wall or of the interventricular septum can result (Schuster and Bulkley, 1979).

Ventricular dilatation following myocardial infarction is not uncommon but the reported incidence varies widely depending on the selection of the study population. In autopsy series the incidence of dilatation post infarction lies between 45% and 60% (Hutchins and Bulkley, 1978; Pirolo et al, 1986). Observational clinical studies in unselected patients with acute myocardial infarction indicate a lower incidence of 40% (Jeremy et al, 1987; Jeremy et al, 1989). Two groups, restricting patient selection to those with transmural infarction, report widely differing incidences of 28% (Eaton et al, 1979) and 55% (Warren et al, 1988). If assessment is limited to cases of anterior transmural infarction, the incidence is higher at

57% (Erlebacher et al, 1984). However frank aneurysm formation, an extreme form of remodelling, was only documented in 35% of cases of transmural anterior infarcts (Meizlish et al, 1984). Animal models of acute infarction involving coronary ligation report similar figures to the human study of transmural anterior infarcts with greater than 60% of animals exhibiting ventricular dilatation (Hochman and Bulkley, 1982; Weisman et al, 1985).

REMODELLING: RISK FACTORS AND POSSIBLE MODIFICATION

1) Site and size of infarction

Autopsy studies confirm the importance of large and transmural infarcts in the pathogenesis of left ventricular dilatation (Hutchins and Bulkley, 1978; Pirolo et al, 1986). However the relationship between ventricular enlargement and infarct size has not been borne out in all studies. Eaton et al (1979) found no significant difference in infarct size as assessed by peak creatine kinase between those patients who did and did not exhibit ventricular expansion. The site of infarction is also important as dilatation is more common after necrosis in the left anterior descending coronary artery territory (Warren et al, 1988).

Animal models of infarction indicate that there is a threshold for infarct size below which dilatation is not seen. In the dog above this threshold, ventricular dilatation does occur but the extent of enlargement does not correlate with infarct size (Eaton and Bulkley, 1981; Weisman et al, 1985). In contrast investigation using the rat model has demonstrated a correlation between cardiac dilatation and infarct size which may be a consequence of the relatively poor collateral circulation in this species (Hochman and Bulkley, 1982). Animal studies confirm that remodelling is

not seen in nontransmural infarcts (Eaton and Bulkley, 1981).

2) Patency of the infarct related artery

There is evidence from animal studies to indicate the importance of coronary artery patency. Hochman and Choo (1987) demonstrated that reperfusion after 30 minutes in a rat model of infarction results in a reduction in infarct size, in the transmural extent of the infarct and in infarct expansion over the subsequent 2 weeks. If reperfusion is delayed until 2 hours after occlusion then infarct size and the transmural extent of the infarct are not reduced. However late reperfusion does reduce infarct expansion. In a similar study Hale and Kloner (1988) examined the effects of reperfusion after 30 and 90 minutes in a rat model of infarction. Early reperfusion completely inhibits ventricular dilatation and scar thinning. Later reperfusion is followed by some ventricular dilatation but does prevent gross expansion and scar thinning.

In man persisting occlusion of a coronary artery following acute myocardial infarction has proven to be one of the most important risk factors for ventricular dilatation. In an observational study the degree of perfusion of the infarct related artery was the most important predictor of volume change following infarction as assessed by multiple linear regression analysis (Jeremy et al, 1987). Likewise, in a different study, left ventricular function improved over the first two weeks after infarction in patients with spontaneous reperfusion, whereas those patients with persisting occlusion suffered a decline in ejection fraction (Verheugt et al, 1986). The benefit of spontaneous reperfusion was confirmed in another study which in addition found that streptokinase induced reperfusion (within 3 hours of the onset of chest pain) resulted in even better

preservation of ventricular function (De Feyter et al, 1983).

Over the last 10 years there have been a large number of placebo controlled studies of thrombolytic agents. The more detailed have included coronary angiography and as a result patency of the infarct related artery has been identified to be associated with both improved preservation of ventricular function and prevention of ventricular dilatation (Touchstone et al, 1989; Lavie et al, 1990). In addition some studies were able to demonstrate a lower mortality in those with successful reperfusion (Kennedy et al, 1985; Dalen et al, 1988). Despite all this evidence supporting the importance of a coronary artery patency, there is one study which failed to show prevention of ventricular dilatation following successful thrombolysis (Warren et al, 1988). This was a small study in which thrombolysis was administered relatively late after the onset of pain and on its own does not nullify the evidence that reperfusion is valuable.

The larger placebo controlled trials of thrombolytic therapy clearly demonstrate a lower mortality in the groups receiving active treatment (GISSI, 1986; Kennedy et al, 1988; Meinertz et al, 1988; AIMS trial study group, 1988; Wilcox et al, 1988). Although some of this effect may be a result of reduction in infarct size, at least part of the benefit appears to be mediated by the ability of a patent artery to prevent ventricular dilatation. In particular in the ISIS-2 study there was a significant reduction in mortality in the group treated with both streptokinase and aspirin even when treatment was initiated late, between 12 and 24 hours after the onset of pain (ISIS-2, 1988). It is very unlikely that myocardial salvage with a reduction in infarct size would have been achieved by thrombolysis so late in the evolution of an infarct. The most likely explanation is that

aspirin and thrombolytic therapy result in a patent coronary artery that reduces the tendency to ventricular dilatation.

Percutaneous transluminal coronary angioplasty is an alternative method that may be used to remove an occlusive coronary thrombus. However the role of angioplasty in infarction is still under debate. It is clear that there is no place for early angioplasty after thrombolysis if it is only to remove a residual stenosis in a patient without recurring chest pain (Topol et al, 1987; Simoons et al, 1988; TIMI Study group, 1989). Such treatment is associated with an increased incidence of complications and mortality.

Angioplasty for patients where thrombolysis has failed to reopen the occluded artery may be advantageous and may result in an improvement in left ventricular function as demonstrated in a non randomised series by Fung et al (1986). Guerzi et al (1987) assessed the impact of angioplasty 3 days after thrombolysis in a randomised study. Unfortunately the study population was heterogenous as patients were eligible for randomisation if there was a suitable stenosis of greater than 70 % or if the infarct related artery was occluded. Although there was no difference in ultimate resting ejection fraction, the patients undergoing early angioplasty had a significantly greater increase in ejection fraction with exercise. These results are encouraging but the problem remains to be tested in a formal study where patients with myocardial infarction who have persisting occlusion of a coronary artery despite thrombolytic therapy are randomised to either angioplasty or conservative therapy.

Why should a patent coronary artery decrease ventricular dilatation? The

answer to this question has not been resolved, but there are several different theories to consider. Firstly, reperfusion may make the infarcted zone stiffer and more resistant to dilatation as reopening the artery results in intramyocardial haemorrhage, oedema and contraction band necrosis (Althaus et al, 1976; Roberts et al, 1983). In keeping with this theory Force et al (1988) have confirmed that there is an acute reduction in the expansion of an infarct zone in a dog model within 3 hours of delayed reperfusion. Secondly, it has been postulated that the quality of scar tissue is better when preceded by reperfusion. However this was not borne out in a study of rabbit myocardial scar tissue which found no difference in the tensile strength of the scar between infarcts that had and had not undergone reperfusion (Connelly et al, 1985). A third theory suggests that reperfusion is advantageous as it accelerates healing and removal of necrotic tissue. This is supported by evidence from experimental infarction in pigs where reperfusion resulted in more rapid removal of necrotic tissue and more rapid appearance of granulation tissue (Althaus et al, 1977). Braunwald (1989) has suggested that an open artery may provide a viable vascular scaffolding that might limit expansion. Lastly, reperfusion may exert its effect by improving viability and function of the remaining epicardial rim of myocytes (Pfeffer and Braunwald, 1990). These myocytes might then be able to oppose dilatation in a similar way to recovery of hibernating myocardium after revascularisation.

3) Drugs that influence infarct healing

The greatest change in ventricular volume occurs in the early days and weeks following myocardial infarction while the infarct is healing. It is not surprising that drug therapy that delays or inhibits this process can have

deleterious effects. A case report and a clinical study suggest that corticosteroids are harmful after infarction predisposing to aneurysm formation, increasing infarct size and increasing the incidence of ventricular dysrhythmias (Bulkley and Roberts, 1974; Roberts et al, 1976). In a similar way Boden and Sadaniatz (1985) report a possible relationship between ibuprofen therapy (a non steroidal anti inflammatory agent) and the incidence of ventricular septal defect complicating myocardial infarction.

Animal studies confirm that both corticosteroids and non steroidal anti inflammatory agents increase the tendency to ventricular dilatation with extensive thinning of the infarct segment. Both Maclean et al (1978) and Hammerman et al (1983a) demonstrated that high dose and prolonged methylprednisolone therapy resulted in these adverse morphological changes. These effects were not seen following a single lower dose injection at the time of coronary artery occlusion. However the collagen content of the scar did not change even after high dose steroid therapy (Hammerman et al, 1983a). Studies of experimental infarction in dogs report that indomethacin given early after coronary occlusion results in marked scar thinning when assessed after either 1 or 6 weeks (Hammerman et al, 1983b; Hammerman et al, 1984). Although there is some evidence that ibuprofen can reduce infarct size (Jugdutt et al, 1980; Romson et al, 1982), it has also been reported to cause scar thinning and dilatation (Brown et al, 1983; Jugdutt, 1985). The evidence concerning ibuprofen is conflicting as Cannon et al (1985) found that ibuprofen did not cause excessive scar thinning even when given to rats for 6 days following experimental coronary artery ligation.

The mechanism of corticosteroid action has been investigated in a rat model of infarction. The detrimental effect is seen early as the excessive infarct thinning does not increase after the third day post coronary occlusion (Mannisi et al, 1987). At the cellular level the infarct zone is seen to thin as a result of excessive slippage of necrotic myocytes. Steroid therapy reduces the influx of phagocytes into the necrotic myocardium and results in a significant delay in the removal of the necrotic myocytes which Kloner et al (1978) term 'mummification'.

4) Ventricular loading conditions

Infarct thinning and ventricular dilatation can be aggravated by increasing the afterload. In an isolated heart acute diastolic expansion during ischaemia is more marked in association with a high afterload and is reversed by reducing the afterload (Nicklas et al, 1983). Aortic banding to increase intraventricular pressure and induce hypertrophy is associated with increased infarct expansion following coronary occlusion in rats (Nolan et al, 1988). However the same research group has previously reported conflicting results showing that aortic banding reduces expansion after infarction (Weismann et al, 1984). In dogs pharmacological intervention with methoxamine, an alpha adrenergic agonist, increases the afterload and results in greater expansion and thinning of the infarct zone (Hammerman et al, 1985). Similarly, clinical investigation confirms that increased arterial pressure and increased systemic vascular resistance are risk factors for ventricular dilatation after infarction (Pierard et al, 1987).

Physical exercise is an alternative and physiological method of increasing cardiac work. Cardiac output and systolic blood pressure increase during

exertion. Regular exercise produces left ventricular hypertrophy with an increase in muscle mass (Pfeffer et al, 1978). There are conflicting reports concerning the effects of exercise on the myocardium during the healing phase after myocardial infarction. Some animal studies show evidence of thinning and infarct expansion following regular exercise (Sutton and Davies, 1931; Hammerman et al, 1983c; Kloner and Kloner, 1983), while other studies fail to show any detrimental effects (Thompson et al, 1973; Hochman and Healy, 1986). It is relevant that in a clinical study a low level exercise training programme produced detrimental dilatation following transmural anterior infarction if the left ventricle had significantly impaired function (defined in this study as asynergy greater than or equal to 18%) (Jugdutt et al, 1988a). Thus there is reason to suspect that in some patients the additional stress of an exercise programme may be harmful during the healing phase.

VASODILATOR THERAPY

Vasodilator drugs have been used to reduce the forces that drive ventricular dilatation either by decreasing preload, afterload or both. Most studies have employed the nitrate vasodilators or angiotensin converting enzyme inhibitors.

a) Nitrate vasodilators

There is compelling evidence that intravenous nitroglycerin therapy in the early hours following acute myocardial infarction reduces the tendency to infarct thinning and dilatation (Jugdutt and Warnica, 1988b). The benefit in this study was seen despite the mean duration of treatment being only 39 hours. A more recent clinical study confirmed the advantageous effect of nitroglycerin therapy after myocardial infarction on ventricular volumes

both at rest and during exercise (Humen et al, 1989). Randomised mortality studies of intravenous nitroglycerin after myocardial infarction have been performed and the individual results are inconclusive (Flaherty et al, 1983; Jaffe et al, 1983; Jugdutt and Warnica, 1988b). However a statistical overview of all randomised clinical studies found a clear reduction in mortality after intravenous nitroglycerin therapy initiated early after myocardial infarction (Yusuf et al, 1988).

The beneficial results of nitroglycerin therapy may not only result from its haemodynamic effects reducing preload and afterload. Part of its action may be to decrease infarct size and to increase collateral coronary blood flow - both of these being established risk factors for ventricular remodelling. In dog studies intravenous nitroglycerin increases collateral flow after coronary artery occlusion (Capurro et al, 1977) and results in a smaller area of ischaemic injury (Chiariello et al, 1976). The effect of nitroglycerin on infarct size is not dependent on a fall in blood pressure and it is therefore probably related to the increase in oxygen supply from collateral blood flow rather than a decrease in oxygen demand (Jugdutt et al, 1981). Moreover nitroglycerin therapy ceases to be beneficial and may be detrimental if there is excessive hypotension as has been demonstrated in both animal and clinical studies (Jugdutt, 1983; Jugdutt and Warnica, 1988b).

Intravenous nitrates are also able to influence platelet function by inhibiting platelet aggregation. This was demonstrated in 11 patients with angina receiving an infusion of isosorbide dinitrate (De Caterina et al, 1984). More recently Diodati et al (1990) reported that intravenous nitroglycerin significantly inhibited platelet aggregation in 10 patients with

acute coronary syndromes. It seems likely that this effect on platelet function would benefit patients with infarction in a similar way to aspirin.

b) Angiotensin converting enzyme inhibitors

The angiotensin converting enzyme inhibitors are another group of vasodilators that might be beneficial in preventing ventricular remodelling following myocardial infarction. These drugs cause dilatation of the venous and arterial vascular beds and so reduce preload and afterload. The rationale for their use in myocardial infarction is underlined by studies of the response of the renin angiotensin system to this condition. Angiotensin II levels rise following myocardial infarction and reach a peak on the third day (McAlpine et al, 1988). If a patient has heart failure following infarction then the levels are greatly increased (Michorowski and Ceremuzynski, 1983; McAlpine et al, 1988). Elevated levels are even found in patients with asymptomatic left ventricular dysfunction who are not on diuretic therapy (Vaughan et al, 1990).

As a consequence of this increased renin - angiotensin activation both the peripheral vascular resistance and the cardiac work load are increased at a time when the heart is least able to cope with it. As well as increasing the peripheral vascular resistance, angiotensin II is a powerful coronary artery vasoconstrictor (Drimal, 1968) and a positive inotrope (Koch-Wesser, 1965). By increasing the haemodynamic forces and decreasing coronary blood flow increased angiotensin II levels potentiate the tendency to ventricular dilatation.

Animal studies have shown that the angiotensin converting enzyme inhibitor, captopril, is beneficial following myocardial infarction. In a rat

model of infarction involving permanent ligation of the anterior descending coronary artery Pfeffer et al showed that captopril treatment would decrease ventricular dilatation (1985a) and improve survival (1985b). In both these studies the greatest effect was seen in those animals with moderate sized as opposed to large or small infarcts. In animal experiments there was no additional benefit from starting treatment within the first two days of infarction as opposed to delaying the introduction of captopril until 3 weeks after infarction (Pfeffer et al, 1985a; Gay, 1990). However it has been suggested that early captopril therapy following a myocardial infarct might have several advantageous consequences in man. The evidence is reviewed below.

i) Infarct size reduction

Captopril therapy reduces infarct size in some animal models of infarction. Ertl et al (1982) demonstrated a significant reduction in infarct size in captopril treated dogs that had undergone surgical occlusion of a coronary artery. This effect was associated with an increase in collateral blood flow into the ischaemic zone in the treated animals. Infarct size was also decreased by captopril therapy in a pig model of infarction which included a period of reperfusion after 1 hour of ischaemia (De Graeff et al, 1987). The effect was greater following a larger dose of captopril (up to 6 mg/kg/10 min). Other angiotensin converting enzyme inhibitors are capable of infarct size reduction as was seen in a study by Hock et al (1985) using enalaprilic acid. However a beneficial effect on infarct size has not been reported by all investigators. Both Daniell et al (1984) and Liang et al (1982) failed to demonstrate any modification in infarct size as a result of captopril therapy in dogs after permanent occlusion of the left anterior descending coronary artery.

ii) Free radical scavenger

Captopril is a free radical scavenger in vitro (Bagchi et al, 1989a; Chopra et al, 1989; Mak et al, 1990). This property is thought to be related to the presence of a sulphydryl group in its chemical structure as the other non sulphydryl containing angiotensin converting enzyme inhibitors do not have the same activity (Westlin and Mullane, 1988; Mak et al, 1990). Although there is work showing that captopril does not scavenge the superoxide radical (Kukreja et al, 1990), there is general agreement that it is active against the hydroxyl radical (Bagchi et al, 1989a; Mak et al, 1990). Indeed captopril inhibits malondialdehyde formation, a product of free radical activity, in an ischaemic isolated rat heart preparation (Bagchi et al, 1989b).

iii) Limitation of post ischaemic dysfunction (stunning)

There is evidence that captopril limits reversible myocardial dysfunction after a short period of ischaemia, also known as myocardial stunning (Patel et al, 1988). This is of relevance in acute myocardial infarction as there is not a sharp boundary between normal and abnormal myocardium. There is a zone around the infarct that is ischaemic but not necrotic and hence will experience myocardial stunning which may take several days to return to normal function. In 1988 Westlin and Mullane reported that both captopril and a stereoisomer without angiotensin converting enzyme inhibitor activity reduced post ischaemic contractile dysfunction. In addition both agents reduce the incidence of ventricular tachyarrhythmias occurring after reperfusion. These effects were attributed to the sulphydryl group and its proposed free radical scavenging ability. However other vasodilators, enalapril and hydralazine, also improve post ischaemic contractile dysfunction although neither possesses a sulphydryl group



(Przyklenk and Kloner, 1987b). A recent report by Przyklenk and Kloner (1990) indicates that sulphydryl containing and non sulphydryl containing angiotensin converting enzyme inhibitors both reduce myocardial stunning after ischaemia but probably by different mechanisms. The beneficial effect of enalaprilat is blocked by the administration of indomethacin demonstrating that this drug's action is mediated by prostaglandin synthesis (probably PGE_1 and PGI_2). However zofenopril, a sulphydryl containing angiotensin converting enzyme inhibitor, also reduces myocardial stunning but this effect is not inhibited in this way and the authors suggest that free radical scavenging may account for its action.

iv) Modulation of the inflammatory response

There is increasing evidence that angiotensin II is an inflammatory mediator and that inhibition of its production by angiotensin converting enzyme inhibitors has anti inflammatory effects. There are receptors for angiotensin II on human mononuclear leucocytes and tissue macrophages (Shimada and Yazaki, 1978; and Weinstock and Kassab, 1984). Angiotensin II, at least in vitro, increases macrophage binding and phagocytosis of particles via Fc and C3b receptors (Foris et al, 1983). A more recent study has revealed that angiotensin II augments free radical generation in normal human neutrophils (Prabha et al, 1990). Angiotensin II also has an indirect effect on neutrophil function as it causes the release of a chemoattractant from endothelial cells (Farber et al, 1990). Moreover tetrapeptide fragments of angiotensin II have direct chemotactic activity on monocytes (Goetzel et al, 1980).

In light of these actions of angiotensin II, it is not surprising that captopril has anti inflammatory effects in a variety of situations. It inhibits vascular

permeability changes caused by a number of different acute inflammatory mediators (Fantone et al, 1982). In a mouse model of schistosomiasis angiotensin converting enzyme inhibitors decreases granuloma formation (Weinstock et al, 1981; Weinstock and Blum, 1983). Lastly, captopril has been used successfully as an anti inflammatory agent in rheumatoid arthritis (Martin et al, 1984). It is interesting that the molecular structure of captopril is not dissimilar to that of penicillamine which is accepted as an effective drug in the treatment of rheumatoid arthritis.

CLINICAL STUDIES WITH CAPTOPRIL

Captopril therapy following myocardial infarction has been studied in clinical trials, and results show a significant benefit from drug therapy. However the details of the therapeutic protocol and patient selection have varied in these studies with the result that general application of this therapy to all patients with acute myocardial infarction has not been tested.

In 1988, Sharpe et al published the first report that captopril therapy prevents ventricular dilatation in patients with symptomless left ventricular dysfunction. This group selected 60 patients with Q wave infarction that had an echocardiographic ejection fraction of less than 45% but no clinical evidence of heart failure. Trial therapy with captopril 25 mg three times daily, frusemide 40 mg daily or placebo was started 1 week after acute myocardial infarction. No patient in this study received thrombolysis and the percentage of patients treated with aspirin was not recorded in this paper. The captopril treated group showed a decrease in ventricular volumes and an increase in ejection fraction whereas both the placebo and frusemide treated groups suffered ventricular dilatation and a fall in

ejection fraction. These changes were evident at the first assessment (after 1 month). After 3 months the differences were established and they were maintained for the 12 month follow up period. The same group reported similar findings in a second report after the study population had risen to 90 patients (Sharpe et al, 1990).

Pfeffer et al also reported the results of a clinical study in 1988 which examined 59 patients who had been admitted to hospital with their first anterior Q wave infarct and had a radionuclide ejection fraction less than 45% without evidence of overt heart failure. Trial therapy consisted of either captopril (up to 50 mg three times daily) or placebo and this was initiated a mean of 20 days after the acute myocardial infarct. As in the study of Sharpe et al (1988), patients treated with thrombolytic agents were not included. In this study captopril was only of benefit in patients who were at high risk of ventricular remodelling because of persisting occlusion of the left anterior descending coronary artery.

In 1991, Sharpe et al reported a further study which now included patients treated with thrombolytic agents but still restricted eligibility to those with Q wave infarction. One hundred patients were randomised to 3 months treatment with either captopril 50 mg twice daily or placebo. Treatment was started much earlier in this study, between 24 and 48 hours after the onset of ischaemic chest pain. Once more captopril therapy prevented ventricular dilatation in this high risk group. In this trial additional assessment of left ventricular function was made 48 hours after withdrawal of drug therapy to counter the criticism that the previously observed effect was simply due to the acute pharmacological effects of captopril reducing the afterload and preload. It could be argued that 48

hours is still too short a time for all captopril's actions, particularly as an inhibitor of tissue angiotensin converting enzyme, to have vanished (Cohen and Kurtz, 1982). Accepting this limitation, the observed benefit remained after withdrawal of the trial therapy.

A group in Glasgow have published their findings in a two-centre study of 99 patients with acute myocardial infarction (Oldroyd et al, 1991). Patients with acute myocardial infarction were randomised to either captopril 25 mg three times daily or placebo within 48 hours of the onset of chest pain. They were required to have significant ST segment elevation and a Norris score of greater than 3.5. This study also excluded patients receiving thrombolysis. Infarct segment expansion as measured by echocardiographic endocardial segment lengths was less in those treated with captopril. As predicted the effect was most pronounced in patients with anterior infarction.

These studies clearly show that captopril is of benefit in selected patients that are at high risk of ventricular remodelling. In view of the importance of permanent coronary artery occlusion in this process it seems likely that therapy with either thrombolytic agents or aspirin would modify the role of angiotensin converting enzyme inhibitors after infarction. In addition it is not clear if there is any additional benefit or detriment from very early treatment with such an agent.

The general administration of angiotensin converting enzyme inhibitors to all patients following infarction is being assessed in a number of multicentre trials with mortality as the main endpoint (eg ISIS 4, GISSI 3, SMILE, TRACE, AIRE and the Chinese captopril study). Two large

multicentre studies have very recently published their results. Swedberg et al (1992) found that early enalapril therapy did not reduce 180 day mortality in the CONSENSUS II (Cooperative New Scandinavian Enalapril Survival Study) study. Their protocol included all patients with infarction without hypotension. In contrast the SAVE (Survival and Ventricular Enlargement) study found a reduction in mortality in patients treated with captopril therapy after infarction (Pfeffer et al, 1992). The SAVE protocol delayed randomisation to later than 3 days after infarction and carefully selected patients with an ejection fraction less than 40% who did not have evidence of ischaemia. It is evident that further information is required before the role of vasodilators after infarction can be decided.

UNRESOLVED ISSUES

Understanding of the pathophysiology of acute myocardial infarction has grown over the last 15 years and, following large multicentre trials, has led to dramatic changes in the management of this condition. Thrombolytic agents are now widely used in acute myocardial infarction to lyse occluding intracoronary thrombus. Aspirin has become routine therapy both acutely and long term after infarction, at least in part to reduce the rate of coronary reocclusion (ISIS-2, 1988).

The role of angioplasty in infarction is still under debate. There is no doubt that this technique is capable of reducing coronary artery stenoses and recanalising occluded vessels. It is possible that angioplasty may benefit patients who have persisting coronary artery occlusion despite thrombolytic therapy, but this remains to be tested scientifically. In addition the timing of such intervention may be important and requires investigation.

The mechanism by which coronary reperfusion confers benefit is not entirely clear. If the artery is reopened early (<3 hours) then there is considerable evidence that myocardium can be salvaged, but it seems unlikely that this is true for later reperfusion. Ultimate patency of a coronary artery or at least perfusion of a coronary bed appears to be important in preventing progressive ventricular dilatation and the related detrimental consequences.

Reperfusion therapy initially caused great fears of myocyte injury resulting from oxygenated blood entering an ischaemic myocardial bed. Nevertheless there seems little doubt that if reperfusion injury exists, it

causes less damage than would have occurred if the artery had remained closed. It is still not clear if adjuvant therapy might improve myocardial salvage after reperfusion. Animal models employing the free radical scavenger, superoxide dismutase, have been disappointing. Modulation of complement or neutrophil function appears more hopeful as a method of decreasing infarct size. Caution should be emphasised when attempting to reduce the inflammatory response as detrimental scar thinning and expansion may result as has been reported with steroids and non steroidal anti inflammatory agents in experimental infarction.

Ventricular dilatation is a consequence of myocardial infarction which may be amenable to modulation by vasodilator therapy. Certainly there is evidence that selected high risk patients benefit from this type of intervention. The exact role of vasodilators when administered in conjunction with the established therapies for acute infarction remains to be elucidated. In addition vasodilator therapy may have beneficial effects separate from the physical effects of the haemodynamic changes on the process of ventricular dilatation, particularly if it is administered early after infarction. Experimental studies suggest that some vasodilators may reduce infarct size and modulate the acute inflammatory response.

Ventricular rupture is a complication of acute myocardial infarction that may be a consequence of infarct expansion or possibly intramyocardial haemorrhage. There has been debate recently concerning the impact of thrombolysis on this complication. There is evidence to suggest that thrombolysis may reduce the incidence if administered early after coronary occlusion. It is not certain if this therapy might predispose to cardiac rupture by intramyocardial haemorrhage if treatment is delayed.

Clearly there are a great number of unresolved issues which will require further investigation. In addressing the question of how best to limit left ventricular dysfunction after acute myocardial infarction I have examined the following aspects:

1) What is the role of early oral vasodilator therapy in acute myocardial infarction in an average patient presenting with chest pain and ST elevation, who is likely to receive thrombolytic therapy?

2) Is there evidence that early oral vasodilator therapy as used in 1) can modulate either infarct size or the acute inflammatory response?

3) Considering the importance of thrombolytic reperfusion and the acute inflammatory response in dictating infarct size and the propensity to ventricular dilatation, is there evidence of interactions either beneficial or detrimental between streptokinase, reperfusion and the acute inflammatory response?

4) Infarct expansion and/or intramyocardial haemorrhage may be involved in the pathogenesis of cardiac rupture post infarction (including rupture of the interventricular septum). What impact has the advent of thrombolytic therapy had on the presentation of ventricular septal rupture as seen in the Royal Infirmary of Edinburgh over the last 7 years?

Each section is presented with a brief introduction, an outline of the methodology used, the results and subsequent discussion of these results. The final section attempts an overview of these issues and suggests

appropriate changes in therapy that might limit myocardial dysfunction after acute myocardial infarction.

CHAPTER 2

THE ROLE OF VASODILATOR THERAPY IN THE MANAGEMENT OF ACUTE MYOCARDIAL INFARCTION

INTRODUCTION

In selected patients vasodilator therapy has been shown to prevent post infarction ventricular dilatation (Sharpe et al, 1988, Pfeffer et al, 1988, Sharpe et al, 1991). It is not known if this is true for all patients, particularly those treated by thrombolysis.

The aim of this study was to evaluate early oral vasodilator therapy with either captopril or isosorbide mononitrate in an unselected group of patients with acute myocardial infarction and therefore assess their role in the typical infarct patient. Left ventricular size and function were the main endpoints with the clinical outcome being a secondary endpoint.

METHODS

Study Protocol

Patients admitted to the coronary care unit with suspected acute myocardial infarction were eligible for study, if they presented within 24 hours of the onset of typical chest pain, with ST elevation and systolic blood pressure greater than 90 mmHg. A history of previous myocardial infarction was noted. The study was approved by the local ethics committee and informed consent was obtained from all participating patients. Elevated creatine phosphokinase levels were not required prior to inclusion as there is frequently a delay in obtaining the results from clinical chemistry which might have reduced the number of eligible patients. All recruited patients were included in the ISIS-4 pilot study and randomisation between trial therapies was performed by the ISIS office in Oxford.

Trial Therapy

Patients (n = 105) were randomised to one of three therapies:- placebo (n = 36), isosorbide mononitrate 20 mg t.i.d. (n = 33), or captopril 12.5 mg t.i.d. (n = 36). Captopril was initially given as a test dose 6.25 mg and if this was well tolerated the full dose was administered 2 hours later. If patients became hypotensive the protocol permitted omission of a dose or the use of half a tablet. Blood pressure was monitored every 15 minutes for the first 2 hours after administration of the test dose. If the trial medication was tolerated, patients underwent treatment for 28 days. Patients who were withdrawn and stopped treatment prior to completing 1 week of trial therapy did not participate further in the study and therefore did not have assessment of left ventricular size and function.

Imaging

Left ventricular imaging was performed by three different techniques, so as to benefit from the strengths of each. Anatomical information was gained from echocardiography and magnetic resonance imaging whereas left ventricular systolic function was quantified by radionuclide ventriculography. The main assessment of left ventricular size and function was performed at 5 weeks, 1 week after stopping drug therapy, as it is known that the pharmacological actions of vasodilators can influence these measurements. This time interval was selected to eliminate continuing captopril activity, particularly through inhibition of the tissue angiotensin converting enzyme which may persist for some days following cessation of therapy (Cohen and Kurz, 1982). This protocol is in contrast to the original work performed with captopril where vasodilator therapy was not withdrawn prior to assessment of

cardiac size and function (Sharpe et al, 1988, Pfeffer et al, 1988).

Echocardiography

Echocardiography was attempted in all patients at 1 and at 5 weeks after trial entry. Images were recorded on Hewlett-Packard echocardiogram machines (both sonos 100 and 1000) with the patient lying semi supine in the semi left lateral position. Where the patient would comply the recordings were made at end expiration. Quantification was performed on the short axis parasternal images at the level of the mitral valve tips (observer: ADH) (figure 1). The anteroposterior and transverse diameters of the left ventricular cavity were measured at this level using the Hewlett-Packard calculation package. The results are reported as the mean diameter (cm).

Radionuclide ventriculography

Left ventricular ejection fraction was assessed by this radionuclide technique, 5 weeks after study entry. Patients lay supine under a gamma camera (Siemens LEM) which was positioned in the modified left anterior oblique position with caudal tilt to provide good ventricular separation. The camera was interfaced to a Siemen's microdelta computer. The blood pool was imaged after injection of 700 MBq Technetium-99m labelled human serum albumin (Miller et al, 1979). After equilibration, an electrocardiogram gated acquisition of 5 million counts was made. Ejection fraction was calculated using a semi automatic technique (Wathen et al, 1990) by two blinded independent observers (ADH and FT). This technique has an interobserver variation of 1% and an interstudy variation of 2% (Wathen et al, 1990).

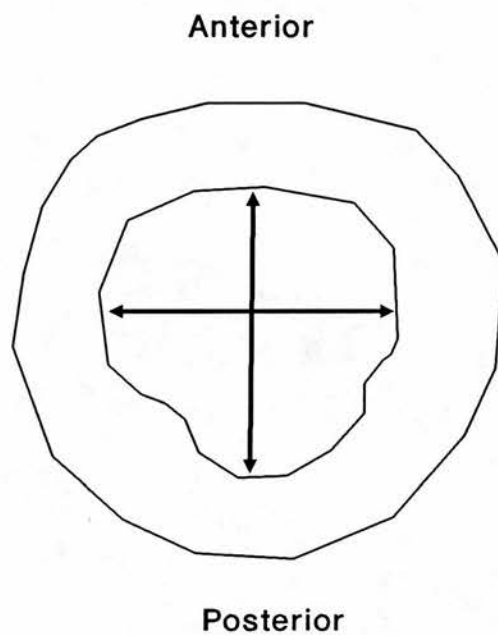


Figure 1 This diagram represents a frame from an echocardiographic study - short axis parasternal view at the level of the mitral valve tips. The internal antero-posterior and transverse diameters were measured using the Hewlett-Packard calculation package and the mean diameter was derived.

Magnetic resonance imaging

Patients were scanned approximately 6 weeks following the onset of chest pain using a low field system (M & D Technology) operating at 0.08 Tesla. A cardiac gating technique was employed to synchronise data acquisition during end diastole. Six or seven slices of 12 mm thickness and separation were required to obtain short axis views of the ventricular chambers from the cardiac apex to the outflow tracts. Two slices with a 32 ms time separation were obtained with each acquisition, which consisted of two averages of 128 frequency encoding steps and 64 phase encoding steps resulting in a pixel size of 3 x 6 mm. This data was interpolated to a 128 x 128 matrix (3 x 3 mm) and smoothed on the final display monitor for image analysis. A double spin echo pulse sequence was used with echo delay times (TE) of 42 and 120 ms for the first and second echoes respectively. The repetition time (TR) was determined by the patients heart rate but if this exceeded 95 bpm data was only acquired every other heart beat. The technical details of the pulse sequence and gating procedure has been described by Smith et al (1986).

The first echo image routinely provided good anatomical detail with clear delineation of the endocardial border. To obtain the left ventricular volume an irregular region of interest was drawn around the endocardial surface on all slices using image analysis software. The values obtained were then summed and multiplied by the slice thickness to produce the total left ventricular volume. Images were quantified by two independent blinded observers (ADH and LWT) and where there was disparity the measurements were repeated by both observers together and a consensus was reached. The interobserver coefficient of variation

using this technique to measure the volume of infarcted muscle has previously been measured using this equipment and was reported by Turnbull et al (1991) to be 10.1%. In addition ventricular wall volumes were calculated from scans obtained on 3 separate occasions in four normal healthy volunteers (Turnbull, 1991). Turnbull found the mean percentage difference in ventricular wall volume measurement was 3% (range 1.1% to 7%). The intraobserver variability for 2 observers was calculated by measuring the same 3 scans on 3 separate occasions and was 6% and 7% respectively. The interobserver variability between Turnbull's observers for ventricular wall volume measurements by mean percentage difference was 14%.

Electrocardiographic analysis

The presence of Q waves and persisting ST elevation was assessed on a 12 lead electrocardiogram performed 24 - 48 hours after the onset of chest pain. A Q wave was defined as an initial negative deflection of greater than 0.04 s duration and 2mm magnitude. ST segment elevation was assessed 0.08s after the J point in the lead with the maximum upward deviation and was deemed to be significant if it was greater than 1mm in the inferolateral leads and greater than 2mm in leads V₁₋₃. Electrocardiographic analysis allowed risk stratification of the study population and a limited sub group analysis.

Statistics

Comparison between groups was performed by the Mann-Whitney U test for continuous variables and chi-square test for the clinical outcome (Minitab release 6.1, Minitab Inc., PA 16801, USA).

RESULTS

Baseline characteristics

The three groups were similar with respect to age, sex, site of infarct and peak creatine kinase (table 2.1). The distribution of patients with previous myocardial infarcts was not even (placebo: 4, isosorbide: 4, captopril: 0). As previous ventricular damage would influence the observations these patients were not included in the assessment of either the left ventricle or the clinical outcome. In view of the concern about the limited and late use of thrombolysis after infarction it is gratifying to note in this study 88% of patients received thrombolysis which was initiated early after the onset of chest pain (mean 3.2 hours). Most patients completed the trial drug therapy.

Reasons for not completing trial therapy (table 2.2)

Eighteen patients did not finish trial medication. Five patients withdrew consent for participation shortly after entry into the study. One further patient in the placebo group withdrew himself after 10 days because of breathlessness, later diagnosed as pulmonary oedema. A minority of patients were withdrawn for minor symptoms e.g. headache or indigestion. One patient developed glomerulonephritis attributed to streptokinase and was withdrawn from trial therapy.

The more serious reasons for withdrawal included reinfarction (1 patient on captopril) and prolonged hypotension with impaired tissue perfusion (3 patients all on placebo). Four patients died within the first 28 days. In the placebo group one died shortly after randomisation and the other died from cardiac rupture at 4 days. Both patients that died in the isosorbide group had cardiogenic shock (one with a history of previous

TABLE 2.1

BASELINE CHARACTERISTICS

| | <u>Placebo</u> | <u>Isosorbide</u> | <u>Captopril</u> |
|-------------------------------|----------------|-------------------|------------------|
| | n = 36 | n = 33 | n = 36 |
| Sex (M : F) | 31:5 | 30:3 | 30:6 |
| Age (yr) | 60.8(8.4) | 60.6(11.2) | 60.3(9.4) |
| Site of ST↑ (Ant : Inf) | 15:21 | 15:18 | 13:23 |
| Previous MI | 4 | 4 | 0 |
| Received thrombolysis | 30 | 30 | 32 |
| Time to thrombolysis (hr) | 3.4(1.7) | 2.9(0.9) | 3.2(1.9) |
| Time to trial therapy (hr) | 13.6(6.3) | 15.3(5.9) | 14.4(6.6) |
| Peak creatine kinase (u/l) | 1429(1152) | 1333(975) | 1494(1178) |
| Completing trial therapy | 27 | 29 | 31 |

Footnote

Continuous variables shown as mean (standard deviation)

TABLE 2.2

REASONS FOR NOT COMPLETING TRIAL THERAPY (n = 18)

| | <u>Placebo</u> | <u>Isosorbide</u> | <u>Captopril</u> |
|--|----------------|-------------------|------------------|
| Consent withdrawn | 2 | 1 | 3 |
| Indigestion | 1 | - | - |
| Headache | - | 1 | 1 |
| Glomerulonephritis | 1 | - | - |
| Reinfarction | - | - | 1 |
| Hypotension with impaired perfusion | 3 | - | - |
| Died | 2 | 2 | - |

Footnote

No patient appears more than once in this table

Data for all patients withdrawn from study shown here including those with previous infarction

myocardial infarction).

Echocardiography

Images were recorded from 89 out of the 105 patients. Image quality precluded quantitative analysis in 17 (19% of those imaged). Ventricular dimensions were compared in the remaining patients who had not had previous infarcts (n = 67).

There was no significant difference at 1 or 5 weeks in mean left ventricular diameter at either end systole or end diastole between the three treatment groups (figure 2). In no group was there a trend to dilatation and even patients in the placebo group tended to decrease left ventricular dimensions during the 4 weeks between studies (table 2.3).

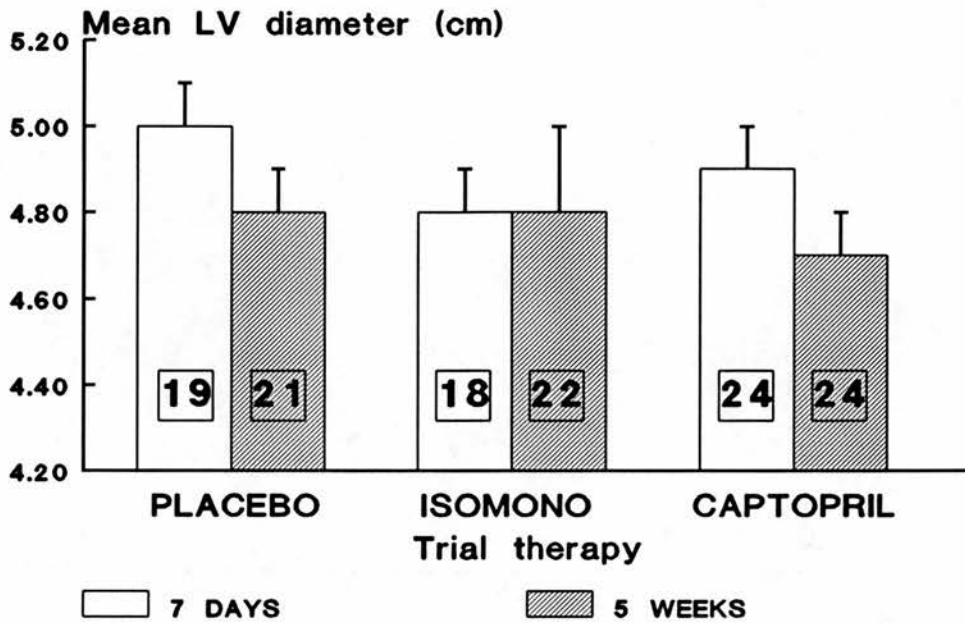
Radionuclide ventriculography

Imaging was performed on 88 patients. Of these 5 were excluded from analysis because of previous infarction. The 17 patients that were not imaged had similar baseline characteristics to the remaining 88 patients except that they had a shorter duration of trial therapy due to withdrawal (n = 13) or death (n = 4).

The left ventricular ejection fraction at 5 weeks was similar in the three groups (figure 3). However, the captopril group had fewer patients with very low ejection fractions (< 20%) (Placebo: 3, Isosorbide: 1, Captopril: 0).

In a post hoc analysis there was evidence of a beneficial effect on ultimate radionuclide ejection fraction from vasodilator therapy

END DIASTOLE



END SYSTOLE

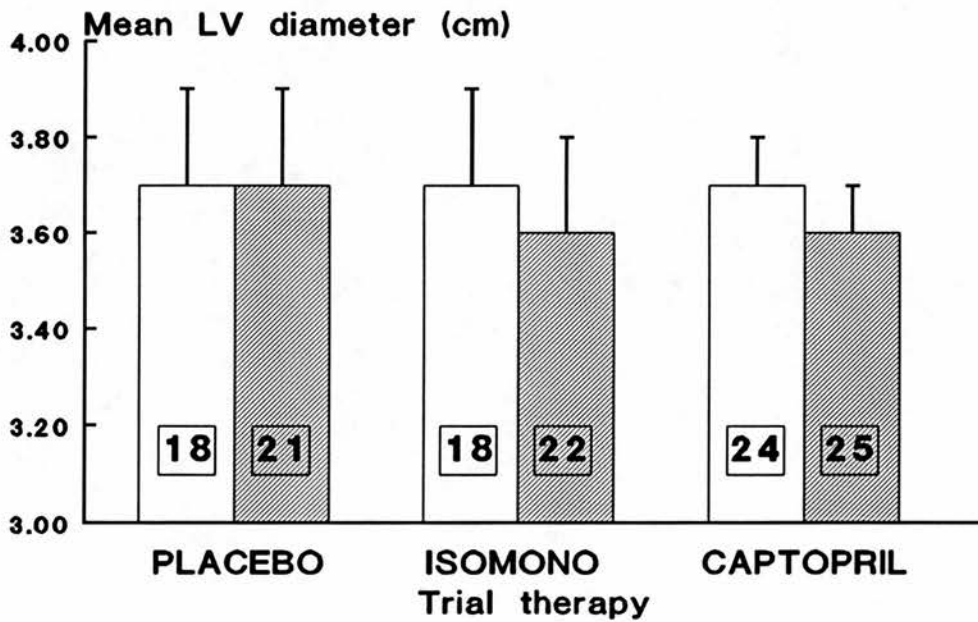


Figure 2 Echocardiographic measurement of mean left ventricular diameter. This histogram shows end-diastolic (top) and end-systolic (bottom) dimensions in the three trial groups at 7 days (open) and 5 weeks (shaded) (mean + SEM). The numbers in boxes indicate the number of patients on which the data is based. There is no significant difference between trial groups.

TABLE 2.3

**DIFFERENCE IN MEAN LEFT VENTRICULAR DIAMETER
BETWEEN 1 AND 5 WEEKS AFTER INFARCTION (cm)**

| | <u>End Diastole</u> | <u>End Systole</u> |
|------------|------------------------|------------------------|
| Placebo | -0.25 (+ 0.15, -0.65) | -0.05 (+ 0.60, -0.50) |
| Isosorbide | -0.10 (+ 0.35, -0.50) | -0.10 (+ 0.2, -0.48) |
| Captopril | -0.25 (+ 0.15, -0.55) | -0.15 (+ 0.15, -0.50) |

Footnote

Differences and 95% confidence intervals calculated by Mann Whitney U test

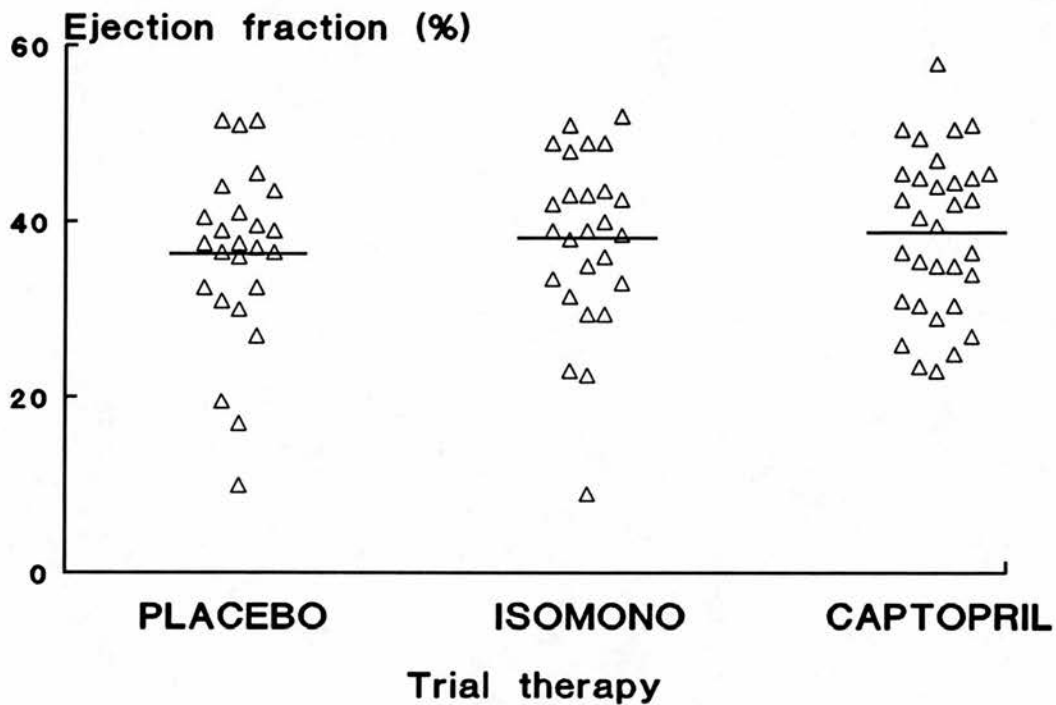


Figure 3 Left ventricular ejection fraction at 5 weeks calculated by radionuclide ventriculography. Each triangle represents 1 patient and the horizontal bars indicate the mean value for each group. There is no significant difference between the three groups (n = 88).

95% Confidence intervals for differences between groups

| | |
|--------|----------------|
| P vs I | - 6.5%, + 3.0% |
| P vs C | - 7.0%, + 3.5% |
| I vs C | - 5.5%, + 5.0% |

(particularly isosorbide mononitrate) in the high risk group of patients with Q waves and persisting ST segment elevation (table 2.4), but interpretation of this data must be cautious in view of the small number of patients in each group and the retrospective nature of the analysis.

Magnetic resonance imaging

Fifty six patients underwent magnetic resonance imaging, the number being limited by machine availability and periods when it was out of service. Four studies were discarded due to poor image quality and three were abandoned due to the patient experiencing claustrophobia. The remaining 49 patients had good quality images that were quantified.

Treatment with vasodilator therapy made no significant difference to the 5 week end diastolic left ventricular volumes (figure 4).

Clinical Outcome

This is an important part of the assessment of the trial therapy as patients who were withdrawn within the first week of the study do not appear in the data on ventricular size and function. Table 2.5 shows the distribution of patients with an adverse outcome. This analysis was based on an intention to treat and as a history of previous myocardial infarction would influence outcome, such patients have been excluded. Two men died and a further two men were withdrawn with cardiogenic shock in the placebo group. These last two patients had ejection fractions measured independent of this study and both were less than 20% (16% and 19%). In addition there was one patient in the isosorbide group who died with cardiogenic shock. These patients missing from the left ventricular analysis are a potential source of bias

TABLE 2.4

LEFT VENTRICULAR EJECTION FRACTION: SUB GROUP ANALYSIS

| | <u>Placebo</u> | <u>Isosorbide</u> | <u>Captopril</u> |
|---------------------------------------|---------------------|----------------------|---------------------|
| <u>Non Q wave infarct</u> (n = 15) | 40.5(10.3) n = 4 | 45.5(6.3) n = 5 | 43.9(10.0) n = 6 |
| <u>Q wave Infarct</u> (n = 68) | | | |
| No ST elevation | 40.6(5.5) n = 14 | 35.6(12.2) n = 10 | 41.4(5.7) n = 15 |
| Persisting ST elevation | 25.1(9.6) n = 7 | 36.9(8.0)* n = 11 | 32.4(9.6) n = 11 |

Footnote

All patients with past history of myocardial infarction were excluded.

Data shown as mean (standard deviation)

* denotes $p < 0.05$ in comparison with placebo

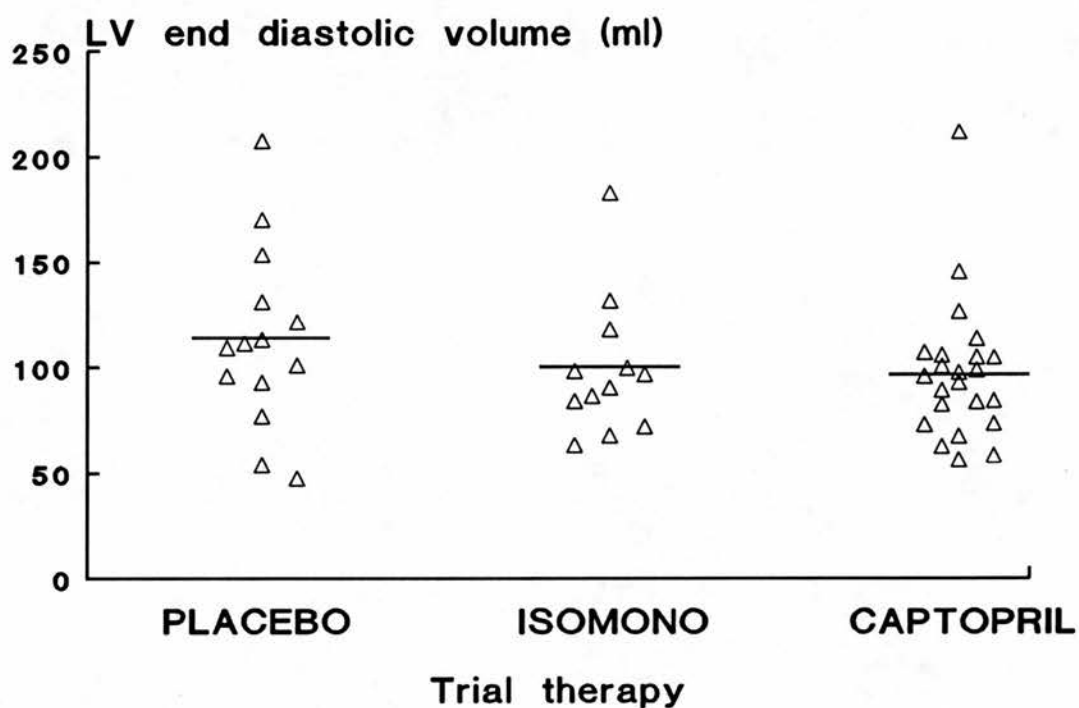


Figure 4 Scattergram showing left ventricular end diastolic volumes at 5 weeks measured by magnetic resonance imaging. The mean has been marked by a horizontal bar. There is no significant difference between the three groups (n=49).

95% Confidence intervals for differences between groups

| | |
|--------|----------------------|
| P vs I | - 15.9 ml, + 33.2 ml |
| P vs C | - 11.2 ml, + 28.6 ml |
| I vs C | - 16.6 ml, + 14.5 ml |

TABLE 2.5

ASSESSMENT OF ADVERSE OUTCOME

| | <u>Placebo</u> | <u>Isosorbide</u> | <u>Captopril</u> |
|-------------------|----------------|-------------------|------------------|
| | n = 32 | n = 29 | n = 36 |
| Died | 2 | 1 | - |
| Cardiogenic shock | 2 | - | - |
| | 4 | 1 | 0 |

P vs C, $p < 0.05$ (Chisquared)

Footnote

No patient appears more than once in this table.

All patients with previous MI have been excluded.

Cardiogenic shock defined as prolonged hypotension with impairment of perfusion of vital organs without evidence of right ventricular infarction.

and should be borne in mind when interpreting the results. In addition it is of interest that no patient in the captopril group had such an adverse outcome. The difference in the clinical outcome between the captopril and placebo group is statistically significant ($p < 0.05$).

DISCUSSION

This study has shown no significant improvement in objective measures of left ventricular size and function from early vasodilator therapy. However captopril may reduce the number of patients with an adverse clinical outcome. This result is in contrast to other published studies reporting the beneficial effect of vasodilator therapy in preventing ventricular dilatation. Sharpe et al (1988) found that the captopril treated group increased their ejection fraction by 5% in the first 3 months whereas in the same time period the placebo group suffered a decrease of 2%. In the study by Pfeffer et al (1988) the left ventricular end diastolic volume increased in the placebo group by 21 mls in contrast to only 10 mls in the captopril group. In addition a two hospital study from Glasgow has recently reported that captopril therapy started within 24 hours of infarction significantly inhibits ventricular dilatation (Oldroyd et al, 1991). The captopril treated subjects experienced an increase in the anterior left ventricular segment length of only 2.8 mm whereas the placebo treated subjects increased by 10.4 mm. Likewise intravenous nitrates have proven advantageous as left ventricular internal end diastolic dimensions increased by 8mm over the first 10 days in the placebo treated patients while there was no significant change in the dimensions of those that received nitrate (Jugdutt and Warnica, 1988b). No patient received thrombolysis in 3 of these studies (Sharpe et al, 1988, Pfeffer et al, 1988, Jugdutt and Warnica, 1988b).

However the study by Sharpe et al (1991) did include patients treated with thrombolytic agents , but only if the electrocardiogram showed a Q wave infarct (Sharpe et al, 1991).

Thrombolysis is likely to have modified the process of ventricular remodelling by reducing infarct size and by decreasing the frequency of persisting coronary artery occlusion. Despite the widespread use of thrombolysis in this study it is relevant that the mean peak creatine kinase was around 1400U/l and that 78% of the patients developed pathological Q waves. Only 10 patients proved to have a normal ejection fraction when assessed 5 weeks after the infarct. This data indicates that the study population suffered significant myocardial damage and that the failure of the study to detect a major benefit from vasodilator therapy does not result from selection of patients with trivial myocardial infarction.

In this study the placebo group showed no tendency to ventricular dilatation, instead the mean left ventricular diameter tended to fall over the 4 week period. This contrasts with the outcome in the placebo groups of the studies by Sharpe et al (1991), Pfeffer et al (1988) and Oldroyd et al (1991) which all reported significant increases in the left ventricular end diastolic dimensions. A likely explanation is that in the majority of patients receiving thrombolysis there is little tendency to remodelling and that vasodilator therapy does not produce additional major benefits in terms of ventricular size and function.

Despite this, vasodilator drugs may have an important role in the management of patients who are at increased risk of infarct expansion

and dilatation. This is borne out from the adverse clinical outcome in our placebo group in comparison to the captopril treated patients. In addition there was evidence of a possible beneficial effect of vasodilator therapy on the subgroup of patients with Q waves and persisting ST segment elevation. Other workers have identified patients with persisting ST segment elevation after thrombolysis as having significantly poorer left ventricular function and a much greater mortality (Barbash et al, 1990). This study is not the only one to suggest that benefit from vasodilator therapy after infarction may be limited to a high risk subgroup. Pfeffer et al (1988) reported a particular benefit from captopril therapy in those patients with an occluded infarct related artery. Within this category the placebo group exhibited dilatation with an increase in end diastolic volume of 31mls as opposed to an increase of only 7mls in the group allocated captopril.

This study is small and therefore of limited statistical power, however it is of similar size to many of the reported studies of captopril therapy following infarction which range from 60 to 100 patients (Sharpe et al, 1988 and 1991, Pfeffer et al, 1988, Oldroyd et al, 1991). In addition statistical calculation shows that it is necessary to randomise only 60 patients in order to detect a 5% difference in ejection fraction (assuming a standard deviation of 12 in the measurements). This study has demonstrated an important finding that should be considered in designing larger studies to assess the role of vasodilator therapy. All patients admitted to a coronary care unit may not benefit from this type of treatment: in particular, those at low risk of ventricular dilatation with small infarcts, inferior territory necrosis and with patent infarct related coronary arteries. In the large multicentre trials (such as ISIS-4) it

may be important to establish which groups of patients benefit and which do not.

CHAPTER 3

THE INFLUENCE OF EARLY VASODILATOR THERAPY ON MYOCARDIAL INFARCT SIZE AND THE ACUTE INFLAMMATORY RESPONSE

INTRODUCTION

If early vasodilator therapy fails to show a large effect on left ventricular dilatation, is there a benefit (as hinted by the better clinical outcome in the captopril group) from other factors. The introduction to this thesis has already described the postulated activities of captopril and the nitrate vasodilators to modulate infarct size and the acute inflammatory response. This chapter details a study that tested for such activity in the patients that were randomised to captopril, isosorbide mononitrate or placebo as described in chapter 2. This was possible as trial therapy was started early after infarction (less than 24 hours after the onset of chest pain), and therefore at a time when these pathological processes might be influenced.

Infarct size was measured by a quantitative tomographic radionuclide technique (Jansen et al, 1985). This method had the advantage of direct measurement of the volume of infarcted muscle 2 to 4 days after the onset of chest pain and therefore many hours after starting trial therapy. Creatine kinase curves were not employed as the peak levels may have occurred prior to initiating trial therapy, particularly in those who were not randomised until 24 hours after the onset of chest pain. In addition the interpretation of enzyme release curves is influenced by thrombolysis as reperfusion is associated with an earlier and often greater peak level.

Two aspects of the acute inflammatory response were assessed- neutrophil activation and free radical activity. Neutrophil activation was quantified using a specific radioimmunoassay for plasma neutrophil elastase (Plow, 1982). As neutrophil elastase is normally found in the

azurophilic neutrophil granules, elevated plasma levels reflect degranulation and the release of enzyme that is associated with activation. It is a reliable indicator of activation that can be readily applied to a large number of samples. In contrast some workers have employed an indirect elastase assay which measures the concentration of peptide B β a product of fibrin(ogen) degradation by elastase (Mehta et al, 1989), but data interpretation is potentially complicated by variations in substrate and inhibitor concentrations. A further approach involves measuring the changes in the flow properties of leucocytes. The ability of leucocytes to pass through a filter in response to a pressure gradient (filterability) decreases with activation. Although diminished filterability correlates well with histological features of activation (Nash et al, 1989), it is a time consuming and again an indirect measure.

The measurement of free radical activity is complicated in vivo and has been discussed at length in the introduction. In this study free radical attack on phospholipid linoleic acid was assayed using high performance liquid chromatography to detect conjugated dienes (Iversen et al, 1985). This index has previously been shown to be elevated after infarction (Bell et al, 1990).

METHODS

Patients

The one hundred and five patients that were randomised in the study described in chapter 2 were eligible for this investigation. Patients were excluded if they were withdrawn from the study within the first 48 hours after randomisation (13 patients: 6 patients withdrew consent in

this period, 1 reinfarcted, 3 had significant hypotension and 3 died). All gave informed consent and the study was approved by the local ethics committee.

Trial therapy

As described in chapter 2 patients were randomised in double blind fashion to one of three treatment groups: double placebo tablets three times daily; isosorbide mononitrate 20 mg three times daily and captopril placebo; or captopril 12.5 mg three times daily with isosorbide placebo. The captopril group received a test dose of active captopril 6.25 mg two hours before starting the full dose regime (the other two groups received a test dose of placebo).

Infarct size

Quantitative radionuclide infarct imaging was performed between 2 and 4 days following the onset of pain and at least 24 hours after entry into the study. Imaging was not carried out in 27 patients because of withdrawal from the study (n = 12), mechanical breakdown (n = 5) and entry into the study before ethical approval for radionuclide imaging had been obtained (n = 10). There was no difference in the baseline characteristics between those who underwent imaging and those who did not.

Two hours prior to imaging, 400 MBq technetium 99m labelled pyrophosphate was injected intravenously. Single photon emission computed tomographic imaging was performed over 360° with 64 images being acquired, each over 10 seconds using a IGE 400 AT maxicamera linked to a Siemens Microdelta computer. Transverse

images were reconstructed by backprojection using a Butterworth filter after correction for centre of rotation and non-uniformity. The images were smoothed twice. The volume of infarcted muscle was measured using a semiautomatic technique that counted the number of voxels with values greater than 65% of peak myocardial uptake (Jansen et al, 1985). One voxel has a volume of 0.24 ml and the results in this chapter are reported in millilitres. It has been shown that this *in vivo* technique correlates closely with infarct size as judged from increased T1 relaxation parameter on magnetic resonance imaging (Turnbull et al, 1991). Images were quantified by two independent observers (ADH and FT). The coefficient of variation between observers was 6.4% and for the same observer was 4.7%. Figures 5 and 6 show examples of an inferior infarct and an anterior infarct with the region of interest drawn around the infarcted area.

Acute inflammatory response

Assessment was by peripheral blood sampling immediately prior to initiating trial therapy and at 6, 12, 18, 24, 36 and 48 hours after the first dose. These times were selected as Bell et al (1990) had already shown that there was evidence of acute inflammatory activation over this period.

a) Plasma neutrophil elastase

Venous blood (5ml) was taken into sodium citrate in HEPES buffer. Plasma was separated in a cooled centrifuge (4°C) and stored at -20°C. Only the top 1 ml of plasma was used for analysis to avoid contamination from the buffy coat.

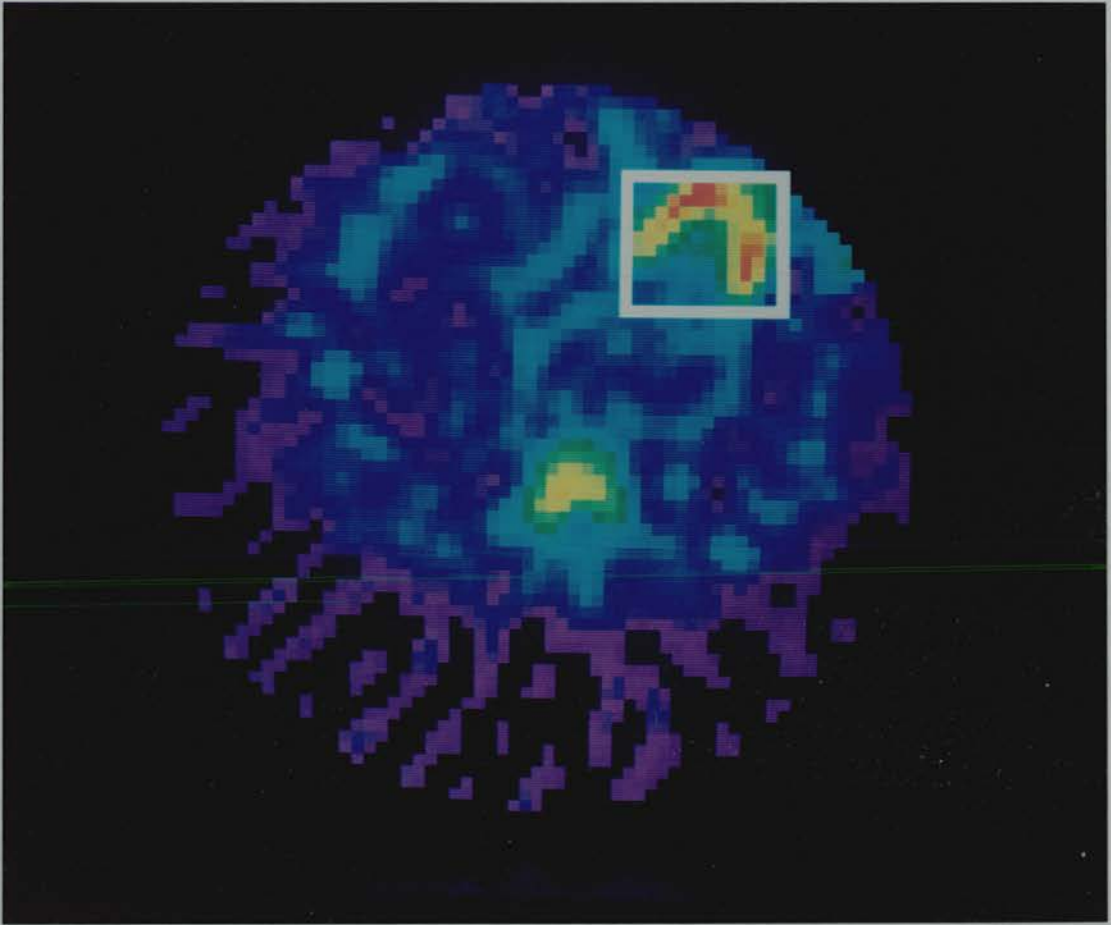


Figure 5 A transverse tomographic image of a patient with an acute anterior myocardial infarct after injection with technetium-99m pyrophosphate. Note the horseshoe shape of the infarcted myocardium. The box has been positioned around the area of infarction to permit quantification.

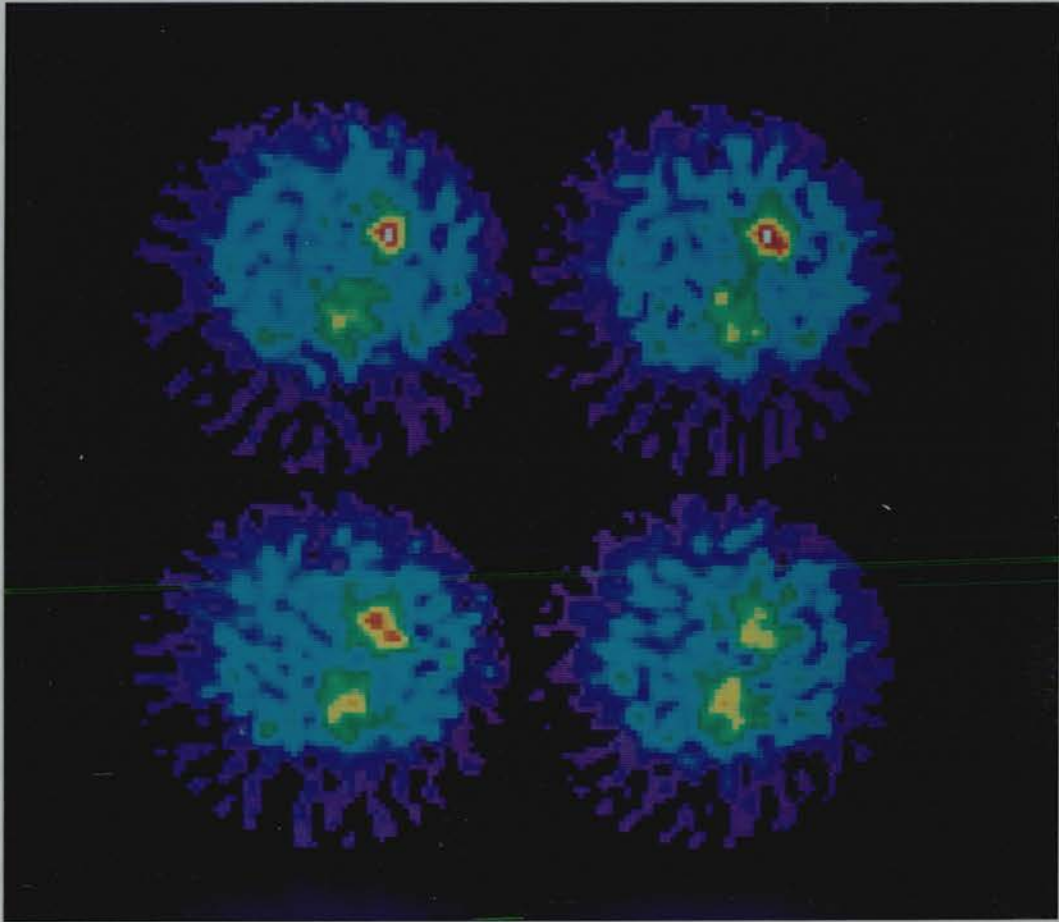


Figure 6 A transverse tomographic image of a patient with an acute inferior myocardial infarct after injection with technetium-99m pyrophosphate. In this image the infarct appears flat like a pancake as the inferior wall lies in the same plane as the slice.

The plasma neutrophil elastase concentration was measured by specific radioimmunoassay with rabbit polyclonal antiserum (Plow, 1982). Purified human neutrophil elastase was obtained from Calbiochem via Novabiochem, Nottingham, UK. The antibody was specific for neutrophil elastase - measuring both free enzyme and that bound to its natural inhibitors. The results are expressed as ng/ml. The intra-assay coefficient of variation was <5% (Bell et al, 1990).

b) Diene molar ratio

A 5 ml sample of blood was drawn into a lithium heparin tube. Plasma was separated in a cooled centrifuge (4°C) and stored at -20°C. The molar concentrations of two isomers of linoleic acid (18:2) were measured in the phospholipid fraction. The native isomer of linoleic acid has double bonds at the 9 and 12 positions (18:2 (9, 12)). Free radical attack can result in isomerisation of this molecule so that the second double bond moves to the 11 position (18:2 (9, 11)) (figure 7). In this form it is said to be a conjugated diene (2 double bonds separated by only one single bond). The measurement of these isomers was performed using high performance liquid chromatography in plasma after enzymatic hydrolysis with phospholipase A₂ and solid phase sample preparation (Iversen et al, 1985). The results are expressed as the molar ratio of 18:2 (9, 11) to 18:2 (9, 12). The ratio increases in the presence of free radical lipid attack. An internal standard (cis 9,11 linoleic acid) was used to compensate for variable loss of sample during the preparation and analysis. The intra-assay coefficient of variation was <3.5%.

Satisfactory assessment of neutrophil elastase concentrations was

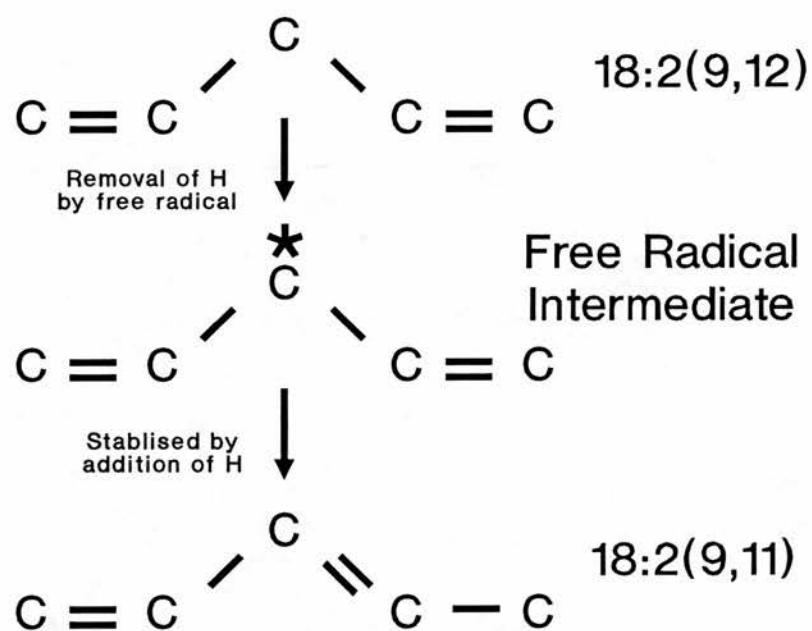


Figure 7 This diagram shows the two isomers of linoleic acid (18:2) and the chemical reaction with free radicals that can result in the isomerisation of 18:2(9,12) to 18:2(9,11).

achieved in 83 patients and of diene molar ratios in 82 patients. The remaining patients were excluded either because they had withdrawn from the study within the first 48 hours ($n=12$) or the samples were either haemolysed or damaged. There was no difference in the baseline characteristics between those that did and those that did not have satisfactory sampling.

Statistics

The effects of trial therapy on infarct size and the acute inflammatory response were analysed using the non-parametric Mann Whitney U test (Minitab, release 6.1, PA, USA). Values of $p < 0.05$ were taken as significant. The significance of the change in plasma markers over the 72 hours after infarction was assessed by analysis of variance (Minitab, release 6.1).

RESULTS

Baseline characteristics

The three groups were well matched at baseline being similar with respect to age, site of infarction, infarct size and time to trial entry (see chapter 2, table 2.1). The initial plasma neutrophil elastase levels (table 3.1) and diene molar ratios (table 3.2) were also similar between the groups.

Plasma neutrophil elastase

Figure 8 shows the plasma neutrophil elastase values in the three groups at various time intervals after the onset of chest pain. Peak levels occurred between 18 and 24 hours after the onset of pain. The peak and average plasma neutrophil elastase levels were elevated in all three

TABLE 3.1

**PLASMA NEUTROPHIL ELASTASE LEVELS (ng/ml) IN RELATION
TO VASODILATOR THERAPY (MEAN \pm SD)**

| | <u>Placebo</u> | <u>Isosorbide</u> | <u>Captopril</u> |
|---------------------------|----------------|-------------------|------------------|
| | n = 28 | n = 29 | n = 26 |
| Pre-therapy | 40.2(16.5) | 53.5(26.2) | 45.0(18.0) |
| Post therapy - peak | 69.4(53.6) | 98.7(84.2) | 62.1(24.0) |
| Post therapy - average | 39.0(18.9) | 52.3(28.5) | 41.6(12.8) |

Footnote

Normal laboratory values: median 18.6 ng/ml (range 9.2-51)

TABLE 3.2

**DIENE MOLAR RATIOS (x 100) IN RELATION
TO VASODILATOR THERAPY (MEAN \pm SD)**

| | <u>Placebo</u> | <u>Nitrate</u> | <u>Captopril</u> |
|---------------------------|----------------|----------------|------------------|
| | n = 26 | n = 27 | n = 29 |
| Pre therapy | 3.27(1.53) | 3.50(1.38) | 3.33(1.87) |
| Post therapy - peak | 3.64(1.72) | 3.78(1.45) | 3.67(1.83) |
| Post therapy - average | 2.80(1.12) | 2.96(1.11) | 2.92(1.43) |

Footnote

Normal laboratory range: median 1.26 (range 0.94-3.03)

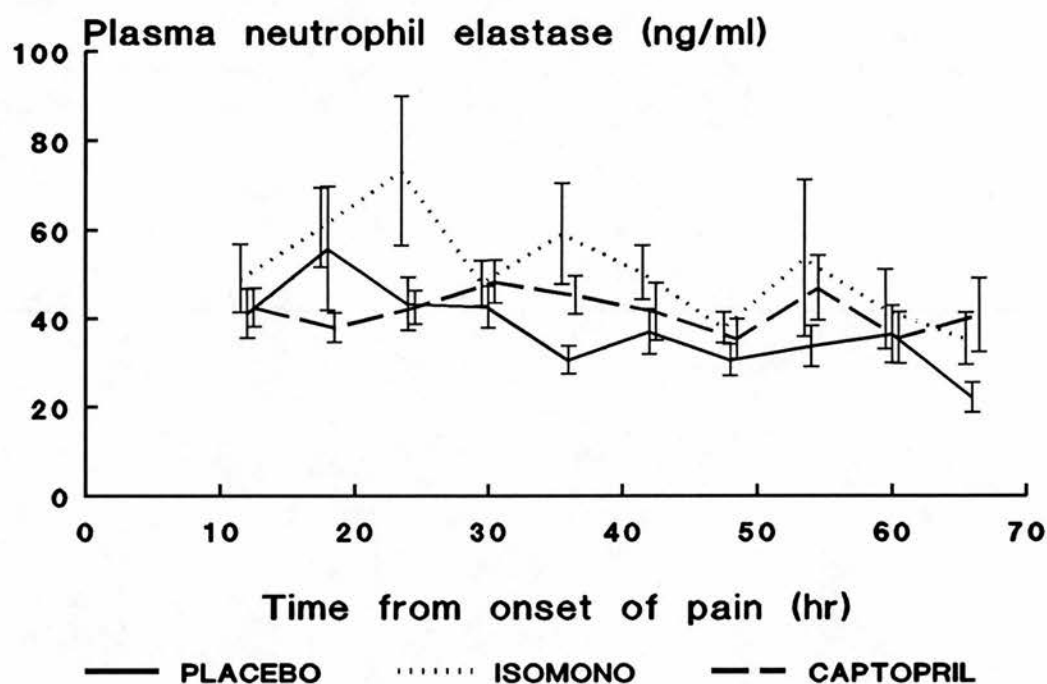


Figure 8 Plasma neutrophil elastase concentrations in 83 patients with acute myocardial infarction with the data (mean \pm SEM) displayed according to the time after the onset of chest pain. The elastase concentrations are elevated above normal but they are not influenced by early treatment with either captopril or isosorbide mononitrate.

groups (normal laboratory values: median = 18.6 ng/ml, range 9.2 - 51.0), but there were no significant differences between any of the treatment groups (table 3.1 and figure 8).

Diene molar ratios

Figure 9 shows the sequential changes in the conjugated diene levels in the three treatment groups after the onset of pain. The peak and average diene molar ratios were also substantially elevated following myocardial infarction (normal laboratory values: median = 1.26, range 0.94 - 3.03) (table 3.2). Peak levels occurred at around 18 hours after the onset of chest pain and there was a significant fall in the diene levels with time up to 72 hours (analysis of variance, $p < 0.05$). Vasodilator therapy did not influence the diene molar ratio (table 3.2 and figure 9).

There was no correlation between plasma neutrophil elastase concentrations and the diene molar ratio. There was a weak correlation between peak creatine kinase levels and both the average plasma neutrophil elastase concentration and the diene molar ratio ($r = 0.25$ and 0.23 respectively, both $p < 0.05$).

Pyrophosphate infarct sizing

Infarct size varied from very small with no significant uptake to extensive infarcts involving much of the myocardium (figure 10). In 9 patients myocardial uptake was diffuse and so quantification of the infarct size by this technique was not possible. The images from the remaining 69 patients were quantified. There were no significant differences in infarct size between any of the groups (figure 10).

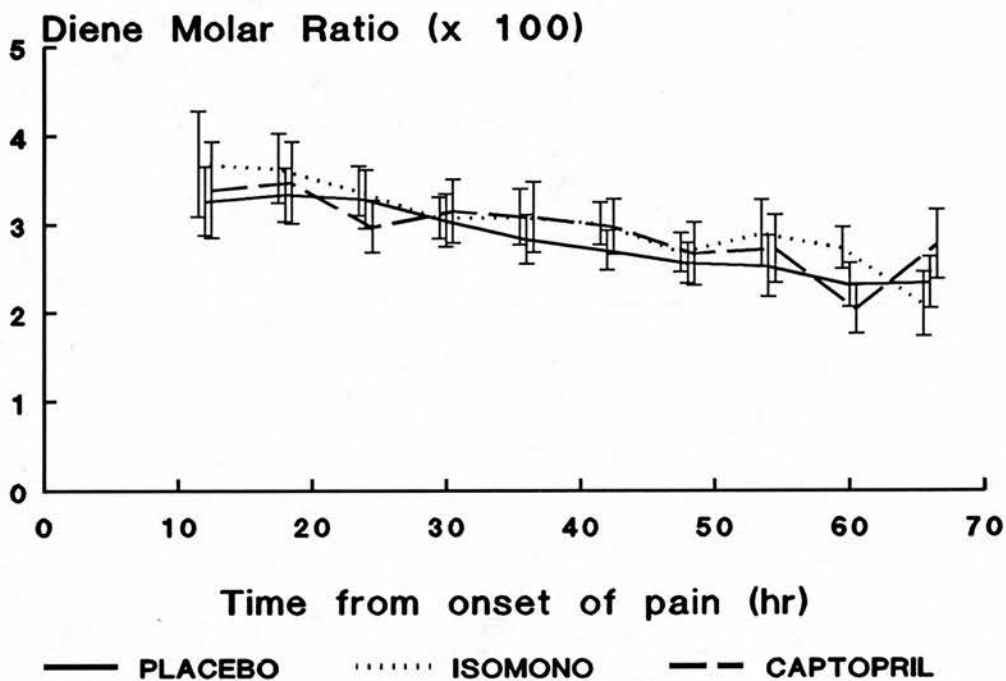


Figure 9 The diene molar ratios (9,11 linoleic acid/ 9,12 linoleic acid) in 82 patients with acute myocardial infarction at various time points after the onset of chest pain (mean \pm SEM) . Peak levels were found at 18 hours and there was a significant fall thereafter ($p < 0.05$). Therapy with captopril or isosorbide mononitrate did not effect the diene molar ratio.

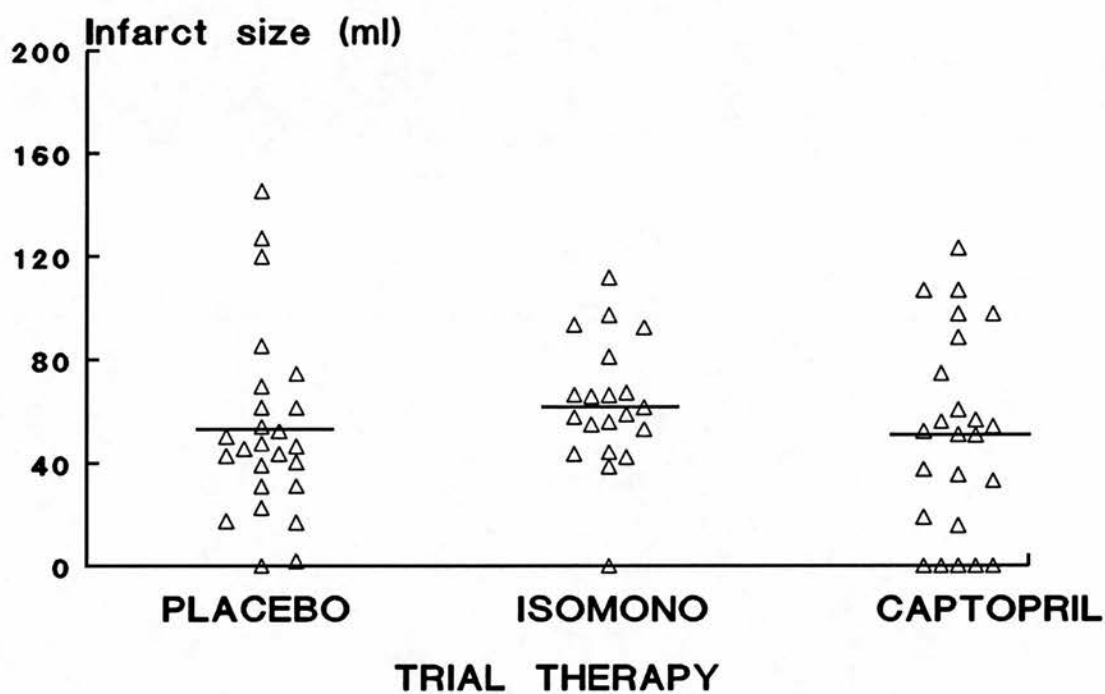


Figure 10 Myocardial infarct size in the 3 trial groups as measured by quantitative tomographic Technetium 99m pyrophosphate imaging in 69 patients. There was no evidence of infarct size modification by vasodilator therapy with either captopril or isosorbide mononitrate.

DISCUSSION

Vasodilator therapy did not modify infarct size in this study. The effect of captopril on infarct size has not previously been assessed in man, but the results of animal studies are conflicting. Ertl et al (1982) reported the beneficial effects of early captopril treatment in an animal model of infarction involving permanent coronary artery occlusion without reperfusion. In a different experimental design, intravenous captopril was given to pigs during a one hour coronary artery occlusion and afterwards during a period of reperfusion producing a dose dependent reduction in myocardial damage (De Graeff et al, 1987). However Liang et al (1982) and Daniell et al (1984) found that captopril did not influence infarct size even when treatment was started 10 minutes after coronary ligation.

Intravenous nitroglycerin has also been reported to reduce myocyte damage in a study of 310 patients with acute infarction (Jugdutt and Warnica, 1988b). Creatine kinase curve analysis showed infarct size to be significantly less in the treated group, particularly when nitroglycerin was started within 11 hours of the onset of pain. In addition animal investigation has shown that nitroglycerin therapy reduces infarct size after coronary occlusion. Intravenous nitroglycerin reduced infarct size in dogs from 12.1% of the left ventricle to 6.4% as assessed 48 hours after occlusion by histological examination (Jugdutt et al, 1981). In a later study, Jugdutt (1985) reported that infarct size was reduced to less than a third of that in control dogs when assessment was delayed until one week after infarction. In addition in this study he showed that there was no detrimental effect on healing and scar formation.

The human study reported in this chapter differs from the previous work as it involves oral medication, initiated 15 hours (mean time) after the onset of chest pain to a group of patients that have largely received thrombolytic therapy. Earlier intravenous administration with larger doses of vasodilators would have been preferable in order to directly compare clinical infarction with the animal models. The main concern of such intervention would be hypotension.

Excessive falls in blood pressure to a mean arterial pressure less than 80 mmHg resulted in significantly larger infarcts in the study of intravenous nitroglycerin reported by Jugdutt and Warnica (1988b). Those with drug induced hypotension had a greater mean infarct size (81.3 gram equivalents (geq)) than a similarly hypotensive control group (65.4 geq). The CONSENSUS II trial showed an increased mortality in those experiencing hypotension after vasodilator therapy (with a systolic blood pressure less than 90 mmHg or diastolic less than 50 mmHg) (Swedberg et al, 1992). The six month mortality was 17% among patients who were hypotensive after enalapril therapy and only 12% among patients who had similar hypotension after placebo. A detrimental fall in blood pressure may have been precipitated by the initial dose of vasodilator being given intravenously as enalaprilat and so inducing a sudden change in the coronary perfusion pressure. Indeed it is relevant that the CONSENSUS II study failed to show a benefit from early vasodilator therapy. A deleterious effect of low blood pressure was also evident in an animal model of infarction used to study intravenous nitroglycerin (Jugdutt, 1983). As thrombolysis per se reduces blood pressure, it is probably desirable to delay initiating vasodilator therapy until after such

intervention unless absolutely necessary. Indeed in most clinical studies, captopril therapy was not given until at least 24 hours after the onset of pain (Sharpe et al, 1988, Pfeffer et al, 1988, Sharpe et al, 1991).

This work confirms previous reports of free radical activity following myocardial infarction (Bell et al, 1990, Davies et al, 1990a). Like the work of Bell et al (1990) the diene molar ratio was highest 12-18 hours after the onset of pain. There was a steady fall in the ratio after 24 hours towards normal levels at 72 hours. The results in this chapter were based on samples taken after randomisation and not immediately on admission to hospital or even prior to thrombolysis. Thus it is not possible to comment on the very early hours after the onset of chest pain or the effect of reperfusion. The exact source of the free radicals causing isomerisation of linoleic acid is not clear. There is considerable animal experimental data (reviewed in the introduction) that indicates that reperfusion after a period of ischaemia results in free radical activity. The next chapter of this thesis specifically addresses this issue employing two markers of free radical activity, conjugated dienes and plasma vitamin C concentrations. The lack of correlation between plasma neutrophil elastase concentrations and the diene molar ratios makes it less likely that activated neutrophils and the stimulated inflammatory response are the main source of the lipid attacking free radicals. Free radicals and their reaction products may be released from dying and necrotic cells and therefore may partly explain the weak correlation between peak creatine kinase levels and diene molar ratios.

Neutrophil activation resulting in elastase release occurs early after myocardial infarction (Mehta et al, 1989; Bell et al, 1990). The data in

this chapter confirms this showing peak values 18 to 24 hours after the onset of pain and a trend to decreasing levels over the following 48 hours. The early peak in elastase concentrations confirms the importance of neutrophil activation in this initial period and agrees with the observation by Bell et al (1987) that radionuclide labelled neutrophils must be reinjected within 24 hours of infarction in order to see reliable myocardial uptake. The standard deviation of the early values is much greater than that of the later measurements. This was also evident in a similar study by Bell et al (1990) that compared patients treated with and without thrombolysis. The large standard deviation is caused by some patients exhibiting a markedly elevated elastase concentration. It is not clear why this occurs. The very early inflammatory response and its interaction with thrombolysis is the topic of the next chapter.

Vasodilator therapy with captopril, had no effect on free radical activity. This differs from animal and isolated heart studies that have shown free radical scavenging by this drug when used in high dose (5 mg/kg) (Westlin and Mullane, 1988, Bagchi et al, 1989b). As discussed above hypotension is a concern early after infarction and this very high dose is unacceptable for use in man so soon after infarction if ever. The dose of captopril used in this study (37.5 mg daily) was small compared to that in the laboratory studies, although not without haemodynamic effects (Flather et al, 1991). The length of time between onset of ischaemic damage and treatment may be an additional explanation of the differing results between animal studies and man as our therapy was started some hours after the onset of chest pain and any attempts to induce reperfusion.

In this study neither captopril nor nitrate therapy influenced plasma neutrophil elastase concentrations. This is perhaps surprising given the known anti-inflammatory effects of captopril and the proinflammatory effect of angiotensin II that were reviewed in the introduction. There have been no previous clinical studies addressing this question in myocardial infarction. Modification of the inflammatory response does not appear to be an important action of vasodilators in myocardial infarction in man, at least using this regime of administration.

This study has demonstrated the lack of influence of early oral therapy with captopril or nitrate on both infarct size and the acute inflammatory response to infarction. In previous clinical studies captopril prevented progressive ventricular dilatation after infarction (ventricular remodelling) even when therapy was not introduced until up to 4 weeks after infarction (Sharpe et al, 1988, Pfeffer et al, 1988). In addition animal investigation has indicated that captopril is equally effective in limiting ventricular dilatation after coronary occlusion whether initiated within 2 days or 3 weeks after the coronary ligation (Gay, 1990). This study therefore provides circumstantial evidence that prevention of the mechanical process of ventricular remodelling may be the most important action of vasodilator therapy after infarction. The optimum timing for the initiation of such therapy remains to be elucidated, but may not need to be introduced until the patient is stable and the risk of precipitous hypotension is decreased.

CHAPTER 4

THE EFFECT OF STREPTOKINASE ON THE ACUTE INFLAMMATORY RESPONSE IN ACUTE MYOCARDIAL INFARCTION

INTRODUCTION

Ischaemic myocardial necrosis stimulates an acute inflammatory response which in turn may increase the extent of necrosis and infarct size. Animal studies (reviewed in the introduction) indicate that free radicals, the complement pathways and neutrophils may mediate such injury. It is not clear if thrombolytic therapy with streptokinase may have advantageous or deleterious interactions with this inflammatory response.

Streptokinase may provoke an inflammatory response as a reaction to coronary reperfusion. There is considerable evidence from animal studies that reperfusion injury exists (reviewed in the introduction). Although myocyte injury from reperfusion has never been demonstrated in man, there is circumstantial evidence to suggest that it may occur. Davies et al (1990a) found elevated levels of malondialdehyde in those with angiographic evidence of reperfusion. Neutrophil activation is evident 2 hours after streptokinase therapy in those that reperfuse (Ranjadayalan et al, 1991) but the early time course of this process has not been defined. Bell et al (1990) found higher concentrations of plasma neutrophil elastase in those patients that had received streptokinase post infarction. A temporal relationship between reperfusion and systemic markers of the acute inflammatory response would provide further circumstantial evidence that such injury might be occurring.

Interactions may occur between streptokinase and the mediators of acute inflammation independent of reperfusion. Firstly, streptokinase is a foreign protein and capable of reacting with anti-streptokinase antibodies that are present in the majority of individuals. In a study of

93 normal volunteers all were found to have IgG anti-streptokinase antibodies (Moran et al, 1984). Indeed type I and III hypersensitivity reactions are well recognised complications of this therapy (GISSI, 1986). Secondly, plasmin is produced in large quantities by the streptokinase-plasminogen complex and this powerful protease may partly activate the complement pathways through direct cleavage of complement components. This reaction has at least been demonstrated in vitro (Ratnoff and Naff, 1967; Taylor and Ward, 1967). Stimulation of the acute inflammatory response by these mechanisms might be detrimental by increasing the degree of neutrophil activation or might be advantageous if a state of relative complement depletion occurred prior to reperfusion.

This chapter describes a study that investigated the time course of the acute inflammatory response in a group of patients that received standard intravenous streptokinase therapy. In view of the risks of coronary angiography early after thrombolytic therapy, a non invasive technique involving ST segment analysis was employed to differentiate those with successful and unsuccessful thrombolysis (Hogg et al, 1988). Free radical activity was measured indirectly by quantification of both plasma conjugated diene levels (Iversen et al, 1985) and plasma ascorbic acid concentrations (Vuilleumier and Keck, 1989). Detailed analysis of the complement pathways was undertaken measuring total activity and the concentrations of individual factors, anaphylatoxins and multimolecular complement activation complexes (Kent and Fife, 1963; Platts-Mills and Ishizaka, 1974; Phimister and Whaley, 1990). Plasma neutrophil elastase levels were analysed to evaluate the extent of neutrophil activation (Plow, 1982).

METHODS

Patients

Seventeen patients were recruited. All were admitted to the coronary care unit with acute myocardial infarction (typical chest pain and ST elevation) and were prescribed intravenous streptokinase 1.5MU by the responsible physician. Subsequent cardiac enzyme analysis confirmed the clinical diagnosis in all patients. The local ethics committee approved the study and all patients gave informed consent.

Blood sampling

Two intravenous cannulae were inserted - one into each arm. The first was used to deliver the streptokinase infusion and the second was used for repeated blood sampling. 5mls blood was drawn and discarded prior to each sample to ensure full evacuation of the dead space. The cannula was flushed with 5mls N saline after sampling to prevent obstruction by thrombus. Blood was removed prior to therapy with streptokinase and 20, 45, 75 and 120 min after initiating treatment.

Conjugated dienes

5mls blood was drawn into a lithium heparin tube (Sterilin) and was spun at 4°C for 10min at 2500rpm. The supernatant was removed and stored at -20°C. The concentration of the conjugated diene of linoleic acid ((18:2) 9,11) in the phospholipid fraction was measured by the method of Iversen et al (1985) (described in chapter 3) and the result was expressed as the ratio of the concentrations of 9,11 isomer to the native 9,12 isomer.

Plasma Ascorbic acid

Blood (5mls) was taken into a tube with potassium ethylene diamine tetraacetic acid (EDTA) anticoagulant and 560 iu Aprotinin (Trasylol, Bayer). Plasma was separated by centrifugation at 2500rpm at 4°C for 10mins. 1ml plasma was added to 1ml 5% metaphosphoric acid before storing the mixture at -70°C prior to analysis. The stock metaphosphoric acid solution was renewed and filtered once a month. Ascorbic acid concentrations were assayed by the method of Vuilleumier and Keck (1989). This depends on enzymatic oxidation of ascorbic acid by ascorbate oxidase and the subsequent generation of quinoxaline from the reaction of dehydroascorbate and 1,2 phenylene diamine to produce fluorescence. The assay was performed on the Cobas-Bio centrifugal analyser. The relationship between fluorescence and the ascorbate concentration was linear up to 200 micromol/l. The assay had a coefficient of variation of 2.7%.

Complement

A further 10mls blood was used for the complement analyses. Half was anticoagulated with potassium EDTA and 560iu aprotinin and half was allowed to clot in a glass tube. It was established that aprotinin did not interfere with the analyses. The concentration of aprotinin was sufficient to inhibit in vitro plasmin production as there was no evidence of complement activation in pre-therapy samples that were spiked with 1500U streptokinase (Kabikinase, Kabi). Plasma, separated by spinning at 2500rpm at 4°C for 10 min, was split into 3 aliquots and stored at -70°C. Serum was pipetted from the glass tube without anticoagulant after clotting and was stored in 3 aliquots at -70°C.

The total haemolytic titration (CH50) was performed using serial dilutions of test serum in conjunction with anti-erythrocyte antibody sensitised erythrocytes according to the method of Kent and Fife (1963). The alternative pathway haemolytic complement titration was also performed using serial dilutions but with unsensitized rabbit erythrocytes which are known to activate the alternative pathway. The method has been described by Platts-Mills and Ishizaka (1974). The levels of individual complement factors were measured by nephelometry with high affinity, monospecific antibodies for C3, C4, Factor B (Atlantic antibodies) and C1 inhibitor (Scottish Antibody Production Unit, Law Hospital, Lanark) (Phimister and Whaley, 1990). The coefficient of variation for the measurement of factor concentrations by nephelometry was 10%. Anaphylatoxin (C3a, C4a, C5a) concentrations were assayed using commercial radioimmunoassays employing specific antisera and I^{125} labelled ligand (Human complement C3a/C4a/C5a des arg (I^{125}) assay systems, Amersham International PLC, Aylesbury). A second antibody was used to precipitate the antibody ligand complexes. The limit of sensitivity was 40ng/ml for C3a, 80ng/ml for C4a and 12ng/ml for C5a. The coefficient of variation was 15%. The concentrations of multimolecular complement activation complexes were quantified using a sandwich enzyme linked immuno adsorbent assay (ELISA). This depends upon the use of two antibodies that have specificity for the activation products of two different components of the complex. The first or "capture" antibody is fixed to a microtitre plate and the second antibody is labelled to permit detection of the complex. The methodology has been described by Phimister and Whaley (1990). The coefficient of variation was 15%. The complexes assessed were C1r:C1s:C1-inhibitor reflecting classical pathway activation, C3bBbP

which is produced by activation of both pathways and C5b-9 complex which indicates terminal pathway activation (see fig 11).

Plasma neutrophil elastase

Using an identical protocol to that described in chapter 3, 5mls venous blood was drawn into sodium citrate with HEPES buffer, before plasma separation. Once again the anticoagulant contained 560 iu aprotinin to prevent in vitro generation of plasmin and possible artefactual neutrophil activation. It was established that aprotinin did not interfere with the elastase assay. The plasma neutrophil elastase concentration was measured by the method of Plow (1982).

Electrocardiographic analysis

Twelve lead electrocardiograms (ECG) were recorded prior to and two hours after initiating the streptokinase infusion. ST elevation, measured 0.08s after the J point, was quantified using the PR segment as a baseline. In accordance with the work of Hogg et al (1988) a change of 50% or more in the lead with the maximum initial ST elevation indicated successful reperfusion. For analysis the study group was split into two groups: those with and those without ECG evidence of reperfusion.

Statistic

Groups were compared using the non parametric Mann-Whitney U test for continuous variables and the chi squared test for frequencies of characteristics. Differences in the response to streptokinase were assessed by calculating the area under the curve for each subject (corrected for baseline values). The areas for the reperfused and non-reperfused groups were compared by the Mann Whitney U test. All tests

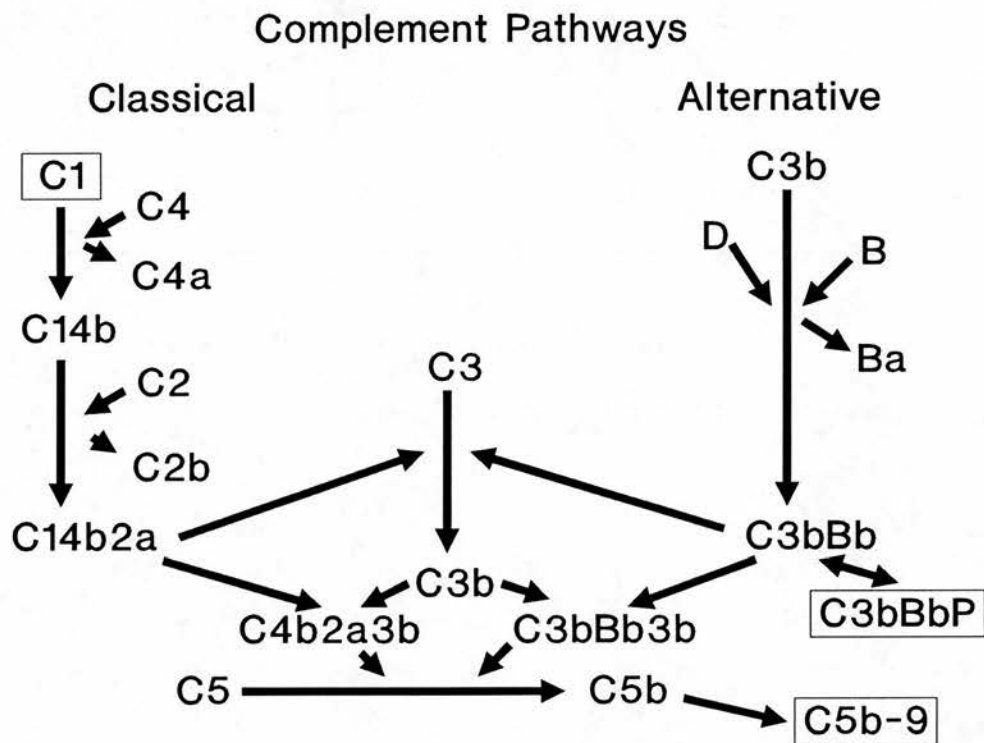


Figure 11 This diagram illustrates the complement pathways. Classical pathway activation generates the complex C1r:C1s:C1 inhibitor. Activation by either pathway causes the formation of the C3 convertase: C3bBbP. Terminal pathway activation is evident by the production of the C5b-9 complex.

were performed by Minitab release 6.1 (Minitab, PA).

RESULTS

Baseline characteristics

The two groups were similar with respect to age, sex, time to thrombolytic therapy and peak creatine kinase (table 4.1). In addition the initial blood pressure and ST segment elevation were comparable (table 4.2 and 4.3). The reperfused group included 6 cases of anterior infarction whereas there were no cases of infarction in this territory in the non-reperfused group.

Blood pressure response (table 4.2)

Both groups suffered similar decreases in systolic and diastolic blood pressure.

Free radical activity

There was no relationship between the duration of chest pain and the initial blood levels of either ascorbic acid or the diene molar ratio (table 4.4). However, even prior to streptokinase treatment the non-reperfused group tended to have higher levels of ascorbic acid and lower levels of the diene molar ratio, suggesting that these patients might have been exposed to less free radical activity than the reperfused group (figure 12). Neither streptokinase nor reperfusion modified the level of free radical activity as indicated by plasma ascorbic acid concentrations or the diene molar ratio (figure 12).

Complement

There was no evidence of complement activation prior to streptokinase

TABLE 4.1

BASELINE CHARACTERISTICS

| | <u>Reperfused</u> n = 12 | <u>Non reperfused</u> n = 5 |
|-----------------------|-----------------------------|--------------------------------|
| Age (years) | 59.5(11.2) | 64.6(6.2) |
| Smokers | 9 | 2 |
| Previous MI | 2 | 1 |
| Diabetes | 0 | 1 |
| Site (Ant:Inf) | 6:6 | 0:5 |
| Time to SK (hr) | 3.7(1.7) | 3.7(2.5) |
| Creatine Kinase (U/l) | 1798(1915) | 1237(402) |

Footnote

Data presented as mean (standard deviation)

TABLE 4.2

BLOOD PRESSURE RESPONSE (mmHg)

| | <u>Reperfused</u> n = 12 | <u>Non reperfused</u> n = 5 |
|---------------------|-----------------------------|--------------------------------|
| <u>Pre therapy</u> | | |
| Systolic | 128(21) | 119(12) |
| Diastolic | 84(13) | 74(11) |
| <u>Post therapy</u> | | |
| Systolic | 103(22) | 100(20) |
| Diastolic | 70(14) | 66(13) |

Footnote

Data presented as mean (standard deviation)

TABLE 4.3

ST SEGMENT ELEVATION (mm)

| | <u>Reperfusion</u> n = 12 | <u>Non reperfused</u> n = 5 |
|-------------------------|------------------------------|--------------------------------|
| <u>Pre therapy</u> | | |
| Lead with maximum | 5.1(3.0) | 3.4(1.7) |
| Total | 23.6(15.7) | 21.2(10.6) |
| <u>Post therapy</u> | | |
| Lead with maximum | 1.3(1.0) | 2.5(0.9) |
| Total | 7(6.2) | 14.9(4.2) |

Footnote

All data presented as mean (standard deviation)

TABLE 4.4

RELATIONSHIP BETWEEN INFLAMMATORY MARKERS AND THE DURATION OF CHEST PAIN

| | <u>Mean</u> | <u>Normal Range</u> | <u>r value</u> |
|------------------------------|-------------|---------------------|----------------|
| Diene ratio | 2.1 | 0.94-3.03 | -.21 |
| Ascorbic Acid (mmol/l) | 3.1 | > 1.96 | -.20 |
| Elastase (ng/ml) | 24.6 | 9.1-51.0 | -.24 |
| CH50 (U/ml) | 284 | 150-250 | -.23 |
| Alt CH50 (U/ml) | 219 | 143-387 | .32 |
| Total C3 (μ g/ml) | 1290 | 720-1800 | -.24 |
| Total C4 (μ g/ml) | 515.9 | 199-574 | -.01 |
| Factor B (μ g/ml) | 266 | 149-421 | -.33 |
| C1-Inhibitor (μ g/ml) | 284 | 160-370 | -.14 |
| C3bBbP (ng/ml) | 8.1 | 10-34 | .11 |
| C1s:C1r:C1-inhibitor (ng/ml) | 8.8 | 4-51 | .22 |
| Sol C5b-9 (ng/ml) | 5.7 | 2-69 | -.34 |

Footnote

No parameter had a significant correlation between pre-therapy value and the duration of preceding chest pain.

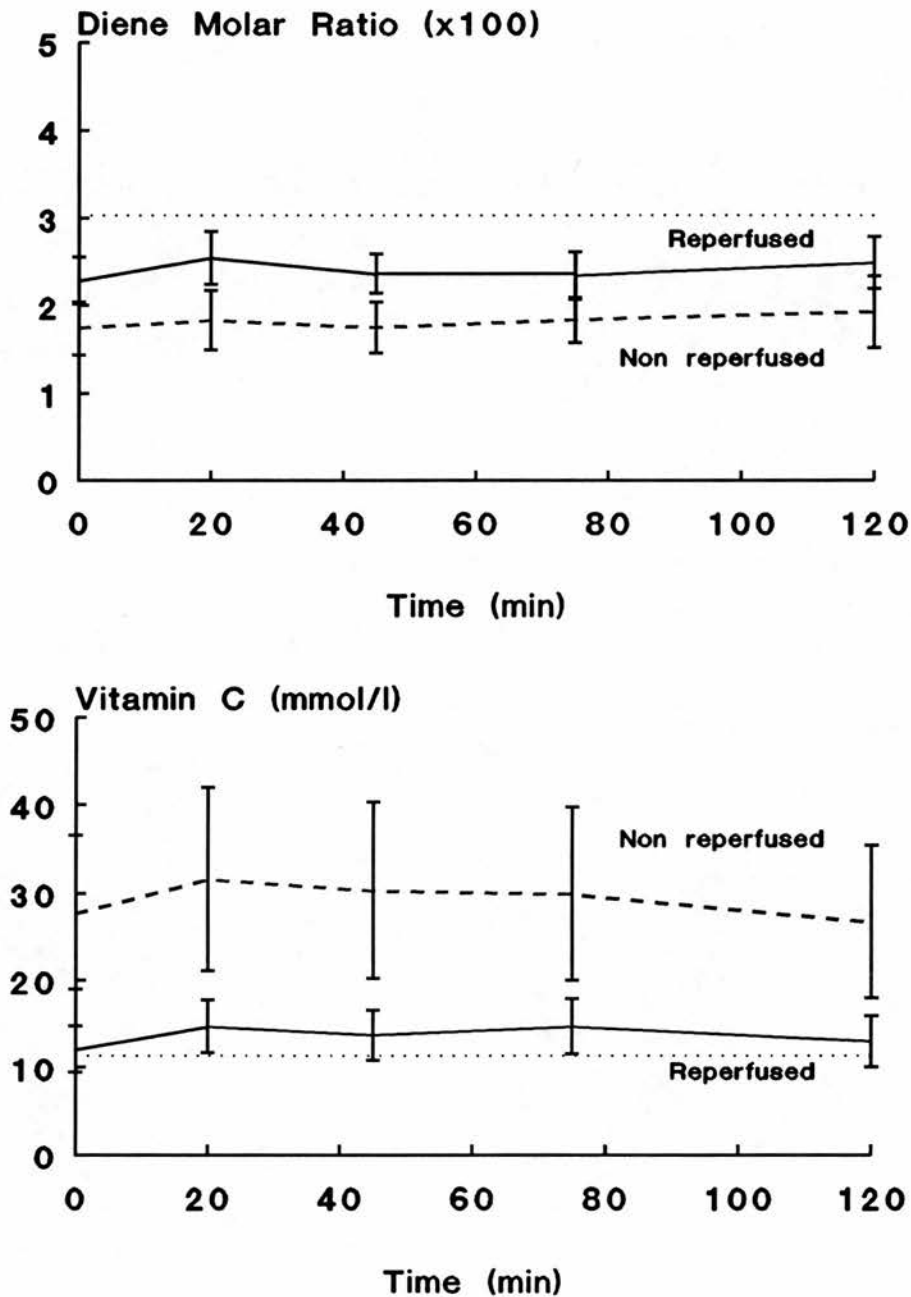


Figure 12 The top graph shows the change in the diene molar ratio with time over the 2 hours after starting streptokinase therapy. There was no significant change in either group. The non reperfused patients ($n = 5$) tended to have a lower level than the reperfused patients ($n = 12$). The bottom graph illustrates the change in plasma vitamin C levels over the same time period. The vitamin C levels did not change in either group of patients. The non reperfused patients tended to have a greater vitamin C level and may reflect a lower preceding level of free radical activity. Mean \pm SEM. The fine dotted lines represent the reference range (upper limit for diene molar ratio and lower limit for vitamin C).

therapy and initial levels were not influenced by the duration of preceding ischaemia (table 4.4). The non-reperfused group suffered a rapid and significant depletion of complement haemolytic capacity, with a fall in classical pathway CH50 from 20 min onwards ($p < 0.05$) (figure 13). There was also a tendency for the alternative pathway CH50 to fall in the non-reperfused group but this failed to reach significance (data not shown). In addition there was an increase in the concentration of the C3b:Bb:P complex at the same time (comparing the 20 minute levels in the two groups, $p < 0.02$) (figure 14). This occurred without a reduction in the systemic levels of C3, C4, Factor B and C1-inhibitor, at least as detected by these antibodies (figures 15 and 16). Neither was there an increase in C1r:C1s:C1-inhibitor complex or C5b-9 complex during this period (figure 17). The patients that did reperfuse did not have evidence of significant complement activation either during or after streptokinase infusion, although there was still a minimal but transient rise in C3b:Bb:P levels detected 20 min after initiating therapy (figure 14). Anaphylatoxin levels rose rapidly after initiating streptokinase therapy and remained high in both groups. This tended to be greater in the patients that did not reperfuse (C3a: $p = 0.15$; C4a: $P = 0.31$) (figure 18). Anaphylatoxin release was limited to C3a and C4a. C5a levels remained low over the 2 hour period in all cases irrespective of perfusion status (data not shown).

Neutrophil elastase

Baseline levels of plasma neutrophil elastase were normal in both groups before thrombolysis and there was no trend to increased activity in cases which had experienced chest pain for longer (table 4.4). In the non-reperfused group neutrophil activation was dramatic and rapid in

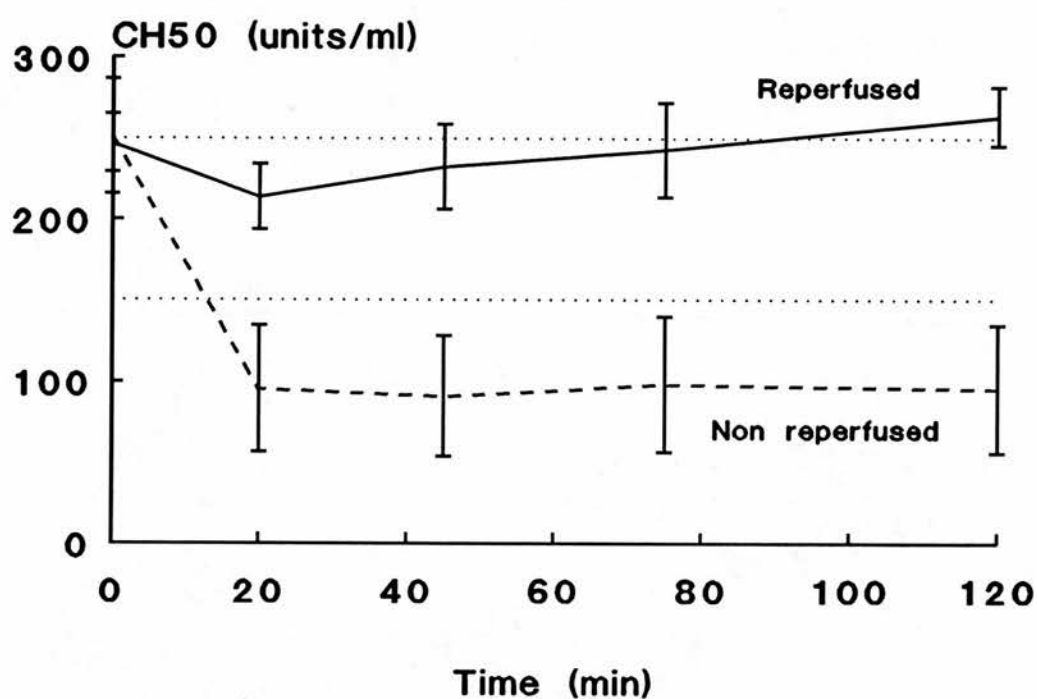


Figure 13 This figure depicts the time course of changes in complement functional activity (CH50) over the 2 hours after starting streptokinase therapy. The non reperfused patients ($n = 3$) exhibited a marked reduction in activity in comparison to the reperfused patients ($n = 9$) ($p < 0.05$). Data only available on 12 patients for this parameter. Mean \pm SEM. The fine dotted lines represent the reference range.

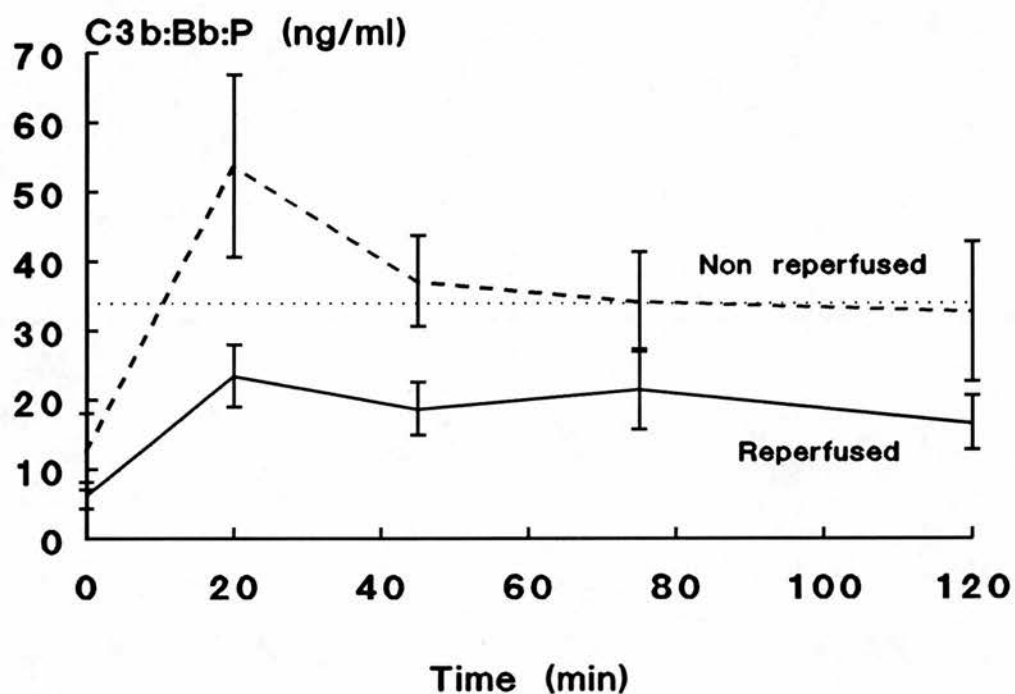


Figure 14 The time course of changes in C3bBbP complex concentrations in plasma after streptokinase infusion. The 20 minute levels are significantly greater in the non reperfused group ($n = 5$) than the reperfused group ($n = 12$) ($p < 0.02$). Mean \pm SEM. The fine dotted lines represent the reference range.

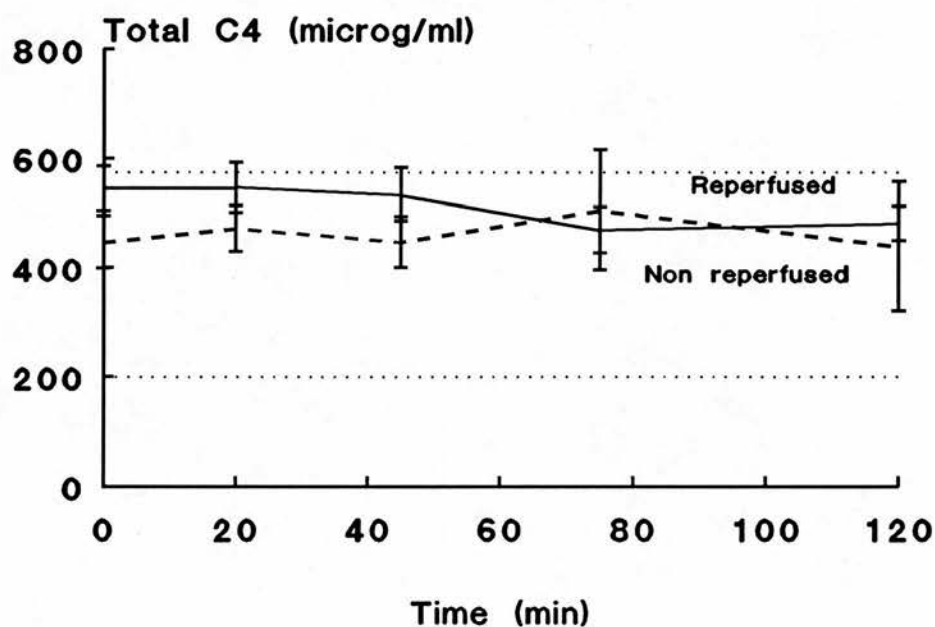
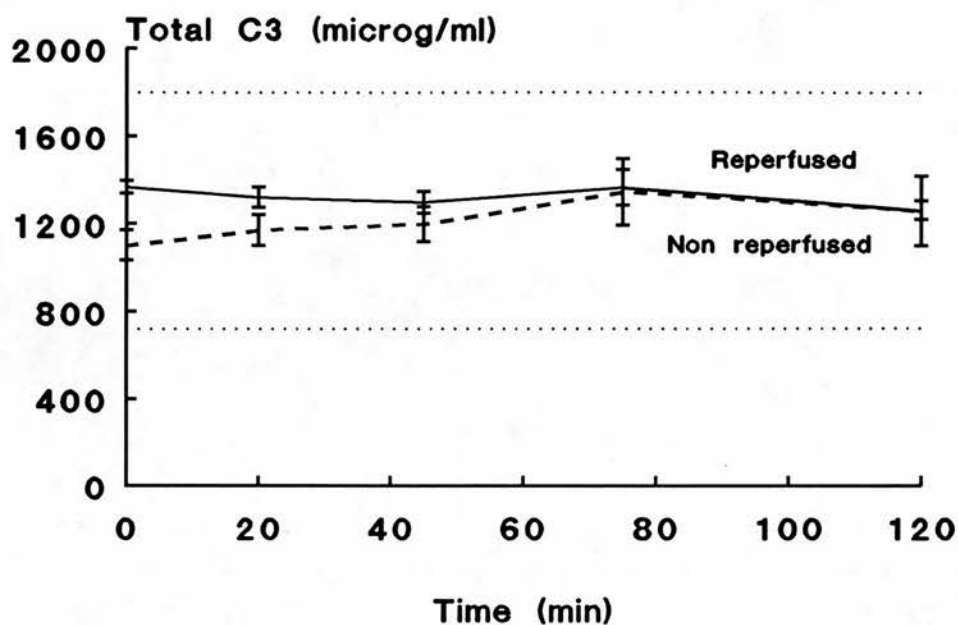


Figure 15 This figure illustrates the lack of change in the concentrations of either total C3 (above) or total C4 (below) in both the reperfused patients ($n = 12$) and the non reperfused patients ($n = 5$) after streptokinase infusion. Mean \pm SEM. The fine dotted lines represent the reference range.

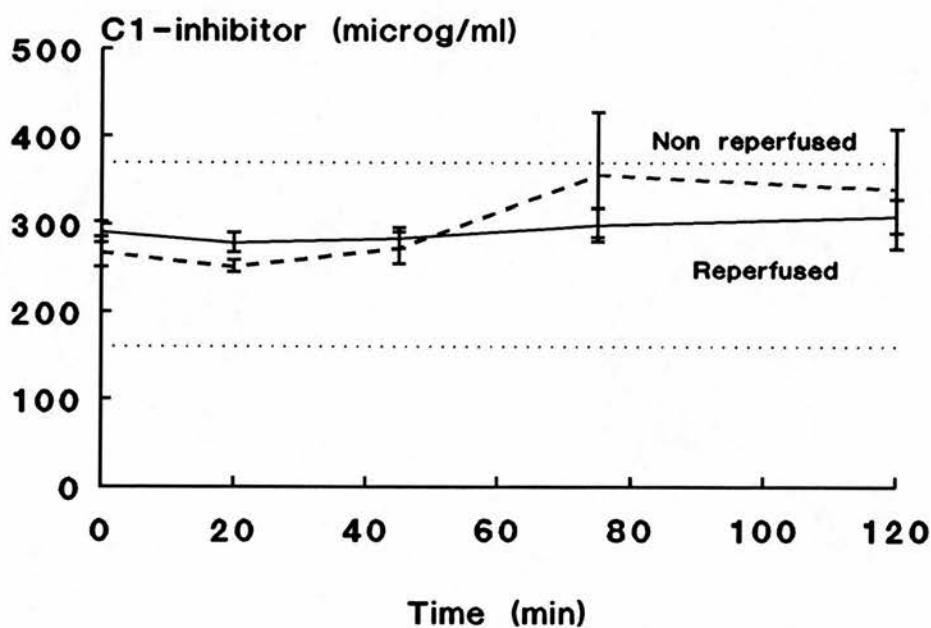
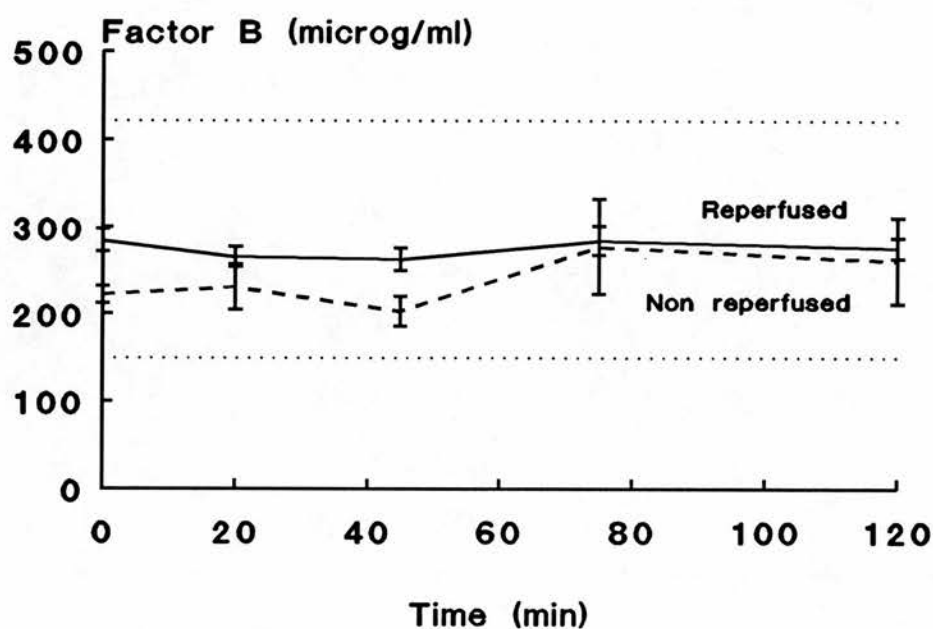


Figure 16. These graphs show factor B (above) and C1-inhibitor (below) levels plotted against time after the initiation of streptokinase therapy in patients who reperfused ($n = 12$) and those who did not reperfuse ($n = 5$). There was no significant change in either group. Mean \pm SEM. The fine dotted lines represent the reference range.

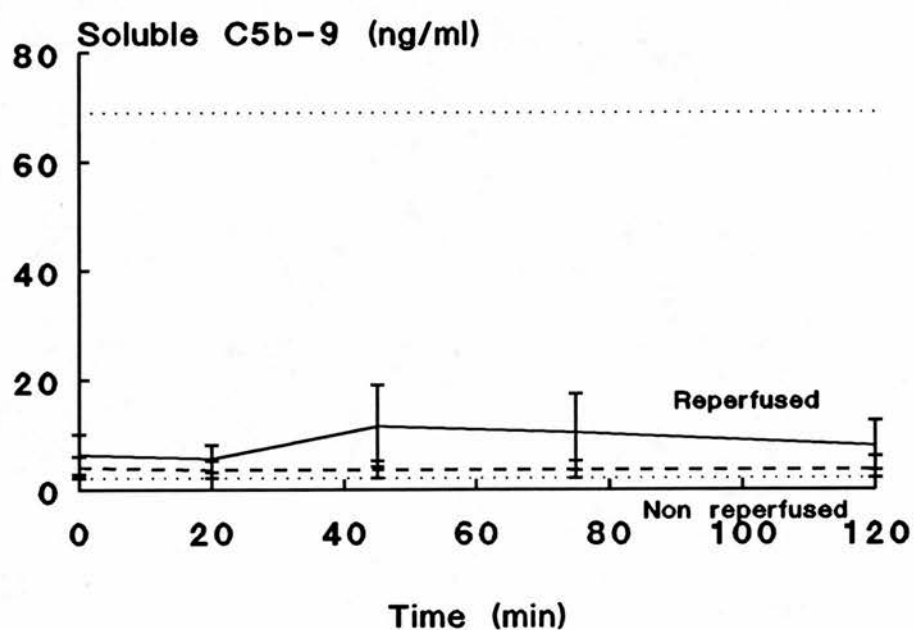
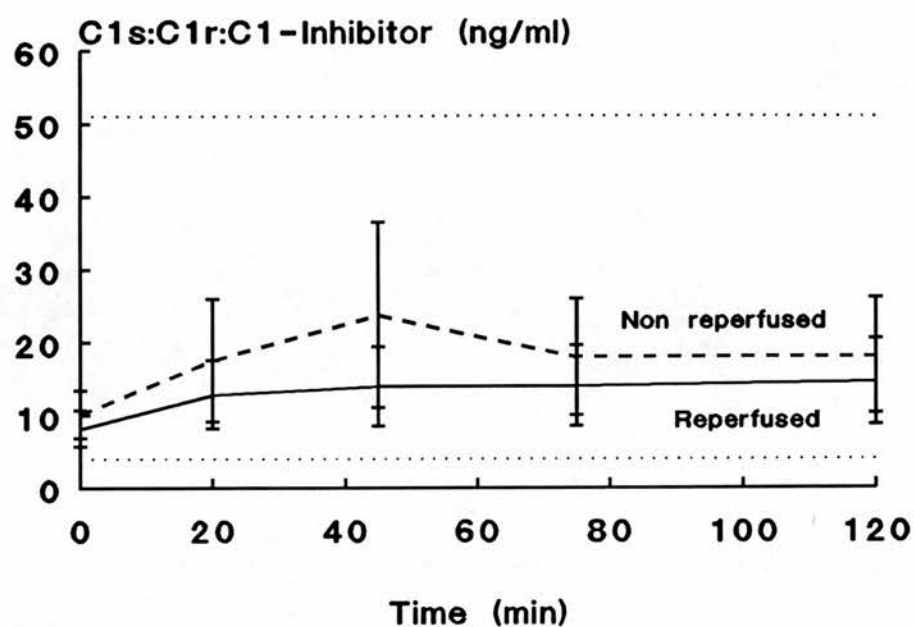


Figure 17. Both these graphs represent changes in the concentrations of multimolecular complexes following streptokinase therapy. There was no change in the level of C1r:C1s:C1 inhibitor in either the reperfused ($n=12$) or non reperfused groups ($n=5$). In a similar fashion the level of C5b-9 remained low in both groups. Mean \pm SEM. The fine dotted lines represent the reference range.

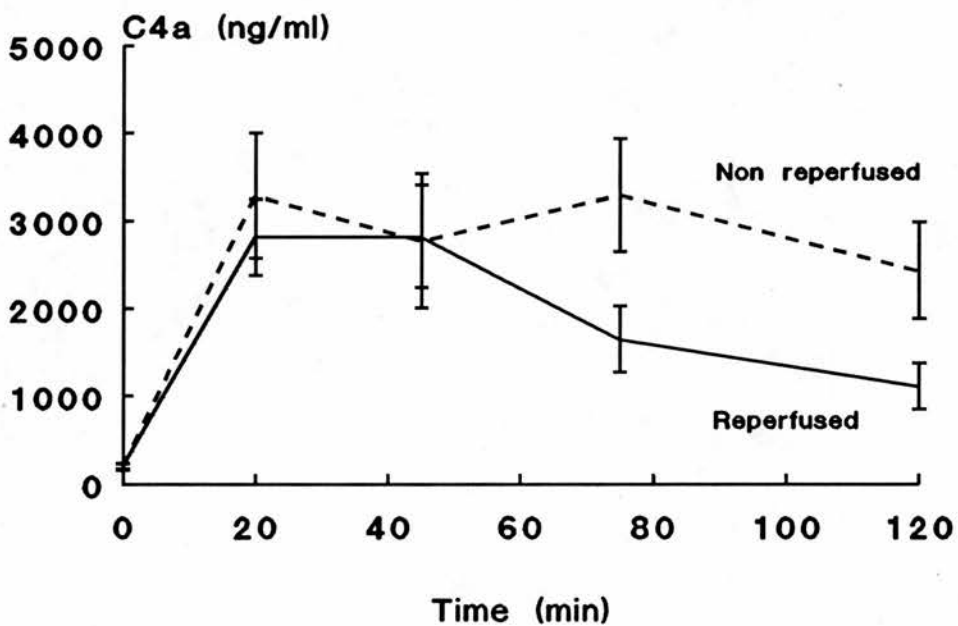
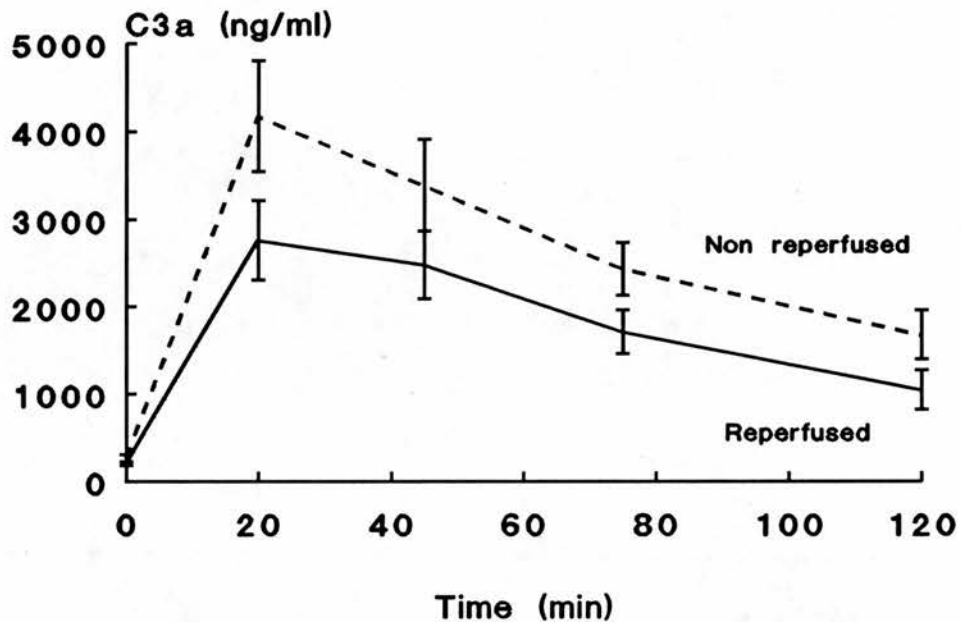


Figure 18. This figure shows the changes in the anaphylatoxin levels after initiation of streptokinase therapy. There is a brisk rise in the level of C3a within the first 20 min. This tended to be greater in the patients without reperfusion ($n = 5$) than those with reperfusion ($n = 12$) ($p = 0.15$). A similar pattern was observed when the C4a levels were measured and once more the non reperfused patients tended to higher values ($p = .31$). Mean \pm SEM. The fine dotted lines represent the reference range.

3/5 cases, starting less than 20 min after thrombolytic therapy (figure 19). In contrast the elastase concentrations tended to rise less in the reperfused group ($p=0.07$) and largely after 45 min, when reperfusion was likely to have occurred after streptokinase therapy.

DISCUSSION

This study demonstrated that complement activation occurred early after starting streptokinase therapy with increased production of the complex C3b:Bb:P and the anaphylatoxins, C3a and C4a. C3b:Bb:P concentrations were significantly greater in those without reperfusion, in whom there was also early neutrophil activation with marked elevation of plasma neutrophil elastase. The increase in anaphylatoxin levels did not differ significantly between the groups. It is relevant that C1r:C1s:C1-inhibitor complexes were not detected as would be expected in a classical antigen-antibody reaction and neither did the levels of soluble C5b-9 complexes or C5a fragments rise which would normally increase with full terminal pathway activation.

The most likely cause of this complement activity is the proteolytic action of plasmin generated by streptokinase plasminogen complexes. Firstly, it is unlikely to be secondary to reperfusion as complement activity is seen early after initiating therapy. Very few arteries regain patency within the first 20 minutes of treatment. In phase I of the TIMI trial only 8% of arteries were patent in the streptokinase group 20 minutes after starting intravenous streptokinase (Chesebro et al, 1987). Indeed most successful reperfusion occurred later than 45 minutes after starting therapy. Secondly, the production of C3b:Bb:P without C1s:C1r:C1-inhibitor and C5b-9 complexes argues against an antigen-

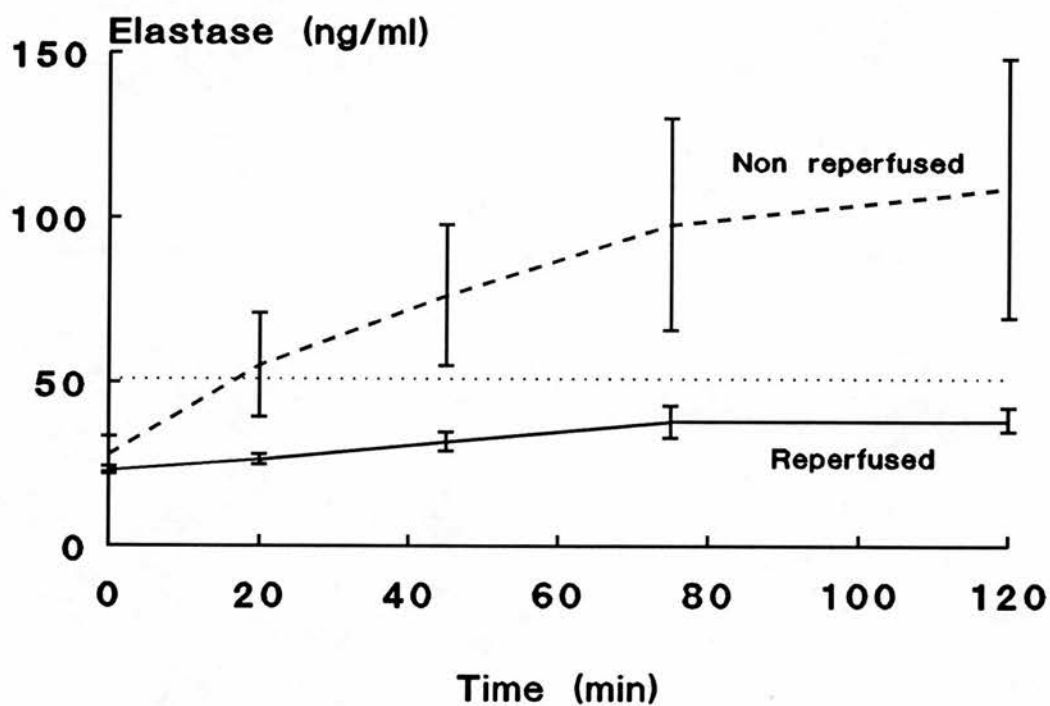


Figure 19 This graph shows the plasma neutrophil elastase concentrations in the two groups over the two hours after streptokinase administration. The non-reperfused patients ($n = 5$) tended to a more rapid and greater elevation in plasma neutrophil elastase levels than the reperfused group ($n = 12$) ($p = 0.07$). Mean \pm SEM. The fine dotted line shows the upper limit of the reference range.

antibody mediated mechanism. This is emphasised by the results from an opportunistic positive control - a patient with active rheumatoid arthritis. She was not given streptokinase but had elevated levels of C1s:C1r:C1-inhibitor complex (79ng/ml), C3BbP complex (137ng/ml) and soluble C5b-9 complex (>200ng/ml). It is well known that complement activation in this disease is related to antigen-antibody reactions. The lack of a decrease in the concentrations of total C3, C4, factor B and C1-inhibitor after streptokinase does not mean that they have not been modified by plasmin. The specific antibodies employed may simply continue to recognise the cleaved product as well as the native factor. Indeed the anaphylatoxin assays demonstrated that significant quantities of peptide fragments were generated in all patients. Perhaps in the non reperfusion group the cleavage products are capable of generating a C3 convertase and so provoking fluid phase complement activation and consumption. In agreement with this theory the increase in C3a concentrations tended to be greater in the non-reperfused group. In addition the functional assays confirmed that complement activity was markedly impaired in those that do not reperfuse. Thirdly, recombinant tissue plasminogen activator, another agent capable of generating plasmin, has been shown to trigger a rise in plasma levels of the anaphylatoxins, C3a, C4a and C5a, within minutes of starting the infusion when used in patients with acute myocardial infarction (Bennett et al, 1987). Unlike streptokinase this agent is not antigenic and therefore the chances of an antigen-antibody reaction are remote. The total levels of C3 and C4 were also measured in their study and remained unchanged during thrombolysis. Unfortunately complement functional activity or multimolecular complexes were not assessed.

An alternative explanation for complement activation after streptokinase could be a low grade antigen-antibody reaction sufficient to stimulate fluid phase amplification by the alternative pathway. Moran et al (1984) have shown that IgG anti-streptokinase antibodies were universally present in normal subjects. Although there is some evidence that anti-streptokinase antibodies can influence reperfusion, their pre-therapy IgG levels do not correlate with successful thrombolysis (Gemmell et al, 1990). There is a relationship between reperfusion and the development of a systemic lytic state, defined as a low plasma fibrinogen concentration after thrombolysis. Bruggemann et al (1990) reported that 83% of patients who had developed a systemic lytic state had reperfused whereas none of the patients without such a lytic state reopened their occluded arteries. Unlike reperfusion itself the failure to develop a lytic state is associated with high IgG anti-streptokinase antibodies (Hoffmann et al, 1988). The correlation coefficient between these two parameters indicates that antibody levels only account for 25% of the variance in the development of a lytic state and therefore other factors are presumably important and might include the interaction of streptokinase with the complement pathways. It is certainly more difficult to link complement activation by plasmin to non-reperfusion. Perhaps full complement activation produces peptides that inhibit the action of plasmin on fibrin and fibrinogen. Separate from the interactions between streptokinase and the plasma proteins it is clear that plaque morphology influences the chances of successful reperfusion. Richardson et al (1989) reported that failed clot lysis was more likely if the culprit plaque was complex (with evidence of a deep fissure, intraplaque haemorrhage or a dissection of the arterial media). Similarly,

Mattfeldt et al (1984) found that all 4 patients that failed to reperfuse in their post mortem study had complex coronary artery lesions.

Neutrophil activation as indicated by elevation of plasma neutrophil elastase concentrations is not evident in the early hours after infarction prior to streptokinase therapy. This is perhaps surprising as other workers have shown evidence of neutrophil activation in acute coronary syndromes, unstable angina and early after acute myocardial infarction. Mehta et al (1989) found that neutrophils from such patients exhibited spontaneous pseudopod formation and granule extrusion. In addition the ability of these cells to produce leukotriene B₄ and to exhibit chemotaxis was decreased suggesting previous in vivo activation. Lastly, peptide B β , a product of elastase mediated degradation by elastase was increased in these subjects, even within 1 hour of onset of chest pain. In a later study Dinerman et al (1990) found that peptide B β levels were highest on admission to hospital and decreased over the following 16 hours. Nash et al (1989) employed white cell filterability, another measure of activation, which was impaired 24 hours after the onset of chest pain and deteriorated further over the ensuing 48 hours. According to this index, neutrophil activation had returned to normal by the 10th day post infarction. Both the study presented in chapter 3 and the work of Bell et al (1990) found elevated concentrations of plasma neutrophil elastase over the 48 hours after the onset of chest pain. However neither study focused on the early hours after presentation. The mean time to thrombolysis in patients studied in this chapter was less than 4 hours. The most likely explanation for the differences between these reports is that neutrophils may progress from mild activation, both before and early after infarction, to more intense

activation over the following 24-48 hours. The variation in the reported time course of activation reflects the variety of different indices employed.

Neutrophil activation was modulated by streptokinase and was most marked in the patients that did not reperfuse. The rise in elastase levels was seen very early after starting the infusion, being apparent even in the 20 minute sample. This neutrophil response was very similar to that seen in the measures of complement activity and it seems likely that it is related to plasmin generated complement anaphylotoxins. There was a non significant positive correlation between the levels of C4a and plasma neutrophil elastase ($r=0.41$). A different time course of elastase release was evident in the patients who reperfused. The rise in elastase levels was less marked and was delayed occurring around the time when reperfusion might be happening. One interpretation is that reperfusion might be responsible for this rise. Ranjadaylan et al (1991) also found an increase in elastase levels 1 hour after streptokinase therapy but despite having angiographic data they do not comment on the response of those without reperfusion. In contrast to the results of chapter 3 and Bell et al (1990), Ranjadaylan's group reported that elastase levels had reverted to normal 6 hours after the infusion. This discrepancy is difficult to resolve as all three studies measured plasma neutrophil elastase. The data in this chapter does explain the large standard deviation seen in the elastase concentrations in the early time points in chapter 3 and in the thrombolysed group in the study of Bell et al (1990). It is likely to be due to excessive neutrophil elastase release in a proportion of the patients that do not reperfuse after streptokinase.

There was no evidence of a change in free radical activity during or after streptokinase infusion using either the diene molar ratio or plasma vitamin C levels as indices. This result differs from that of Davies et al (1990a) who reported increased concentrations of malondialdehyde 2 hours after streptokinase in those with angiographic confirmation of reperfusion. Unfortunately this marker of free radical activity is not specific and may mirror other reactions (Knight et al, 1988). Davies et al also measured the diene molar ratio and were unable to detect an increase in relation to reperfusion. In this respect their data confirms the result reported here. In contrast to both studies Grech et al (1992) found elevated diene molar ratios within 10 minutes of successful primary angioplasty in patients with acute myocardial infarction and no rise in malondialdehyde over the first 48 hours. An animal study showed similar findings with the coronary sinus concentration of 18:2(9,11) rising on reperfusion after 90 minutes of ischaemia (Arroyo et al, 1987). In a recent study in man Purvis et al (1992) reported a significant increase in malondialdehyde concentration and a decrease in plasma vitamin E concentration 90 minutes after starting alteplase (rt-PA) therapy in those who regained artery patency. The use of two different indicators of free radical activity improves the confidence in this result. Despite all these studies the relationship between reperfusion and free radicals remains confusing in man.

It is possible that free radical activity may not be particularly prominent after reperfusion in man. This may be secondary to differences in the rate of reflow between the clinical and laboratory setting. In most experiments reperfusion is sudden whereas in man it is likely to be gradual or even intermittent. This is indirectly illustrated in work by

Krucoff et al (1986) that demonstrated that although ST elevation falls rapidly after successful thrombolysis, it still takes 55 minutes to reach a steady state. Experimental studies in dogs have shown that gradual or "gentle" reperfusion as opposed to sudden total relief of an occlusion reduces post ischaemic damage after cardiopulmonary bypass (Okamoto et al, 1986). Coronary angioplasty with balloon inflation for 60 seconds provides the closest human example of sudden reperfusion after ischaemia. Indeed one group has reported increased levels of free radical activity in the great cardiac vein one minute after balloon deflation (Roberts et al, 1990). Unfortunately this study can be criticised as free radical activity was only quantified by measuring malondialdehyde concentrations. The ischaemic insult of cardiopulmonary bypass has also been investigated as a stimulus for free radical damage during reperfusion in man. Davies et al (1990b) demonstrated that both malondialdehyde and conjugated dienes were produced after reperfusion but the site of generation was not the myocardium. Instead these free radical markers were derived from the extracardiac tissues. Once again this study illustrates the difficulty in establishing a pathological role for free radicals in the heart in man.

It is interesting that the group that did not reperfuse tended to have higher vitamin C levels and lower diene molar ratios. This may reflect a higher background free radical activity in those that reperfuse. One might speculate that those who went on to reperfuse might have undergone a series of spontaneous occlusion-reperfusion cycles prior to the final occlusion that caused admission. This might result in repeated low grade free radical activation and cause these differences.

It was not a purpose of this study to establish whether the observed inflammatory changes were beneficial or detrimental. This issue has been addressed in a number of laboratory studies reviewed in the introduction. Depletion of complement activity might help reduce infarct size in the same fashion as that observed after treatment of laboratory animals with cobra venom factor (Maroko et al, 1978; Crawford et al, 1988) or soluble inhibitory protein CR1 (Weisman et al, 1990). Unfortunately complement depletion was only seen in those that did not reperfuse and was associated with striking neutrophil activation which is likely to act in the opposite direction. Indeed the beneficial effects of neutrophil depletion in animal models of infarction have been reviewed in the introduction. The neutrophil activation occurring in those patients without reperfusion may increase infarct size in those with a poorer prognosis.

In conclusion this study shows significant complement and neutrophil activation early after thrombolysis in those without reperfusion. This is most likely to be independent of anti-streptokinase antibodies. There was some evidence of a modest increase in neutrophil activation in relation to reperfusion. The marked inflammatory activation seen in those patients without reperfusion may be harmful in the early hours after infarction. There is little to support the view that reperfusion enhances the inflammatory response.

CHAPTER 5

ASSESSMENT OF THE IMPACT OF THROMBOLYSIS ON THE PRESENTATION AND PATHOGENESIS OF RUPTURE OF THE INTERVENTRICULAR SEPTUM POST INFARCTION

INTRODUCTION

Thrombolytic therapy is not universally beneficial to all patients. Indeed the data from the last chapter would suggest that it may cause detrimental inflammatory activation in some patients. It has been suggested that thrombolysis may influence or modify the pathogenesis of ventricular septal rupture and other forms of cardiac rupture complicating acute myocardial infarction. Reperfusion can result in a haemorrhagic infarct (Higginson et al, 1982; Waller et al, 1987) and dissection of blood between myocyte bundles might lead to an increased incidence of ventricular septal rupture (Honan et al, 1990) (figure 20). On the other hand there is evidence from autopsy studies in the pre thrombolysis era that ventricular septal rupture follows extreme infarct expansion (ventricular remodelling) with side to side slippage of myocyte bundles, progressive thinning and ultimate disruption of the interventricular septum (Weisman et al, 1988; Schuster et al, 1979) (figure 20). Since thrombolysis decreases ventricular remodelling and reduces infarct size (Touchstone et al, 1989; Lavie et al, 1990) it might also decrease the incidence of septal rupture. Indeed in chapter 2 of this thesis we saw that there was little evidence of ventricular dilatation in a group of patients largely treated with thrombolysis.

Analysis of autopsy data from the GISSI study suggested that streptokinase treatment might increase the risk of cardiac rupture particularly if thrombolysis was delayed until more than 5 hours after the onset of chest pain (Mauri et al, 1987; Honan et al, 1990). Equally, a meta-analysis of 4 trials of thrombolytic therapy indicated that early thrombolysis reduced the risk of cardiac rupture, but that delayed therapy (after 11 hours) might increase the risk (Honan et al, 1990).

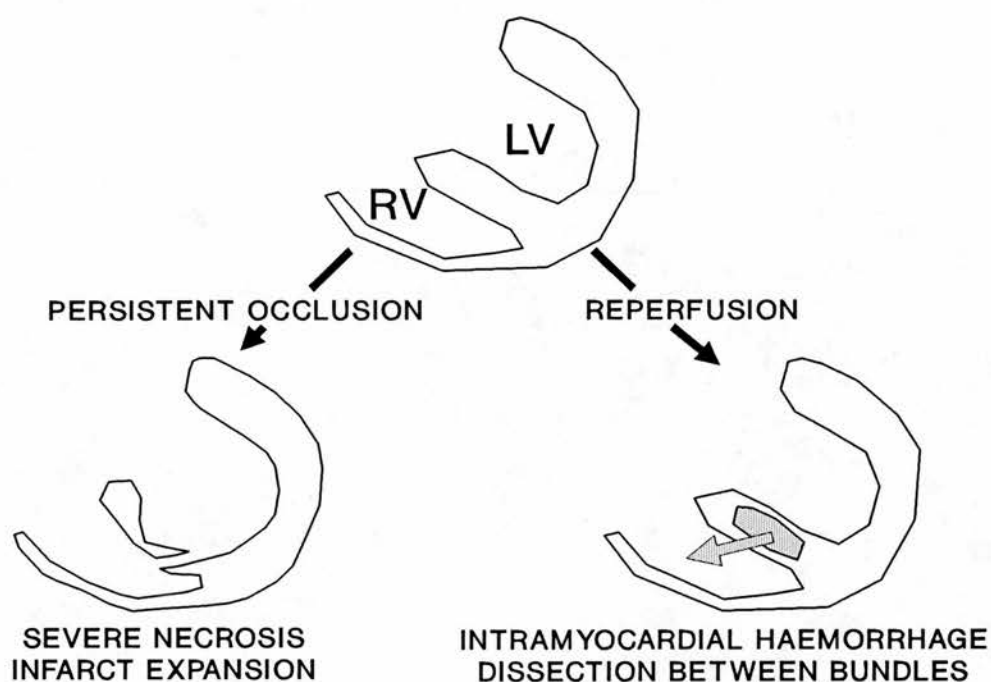


Figure 20 This diagram shows the postulated pathogenetic mechanisms that may underlie the development of ventricular septal rupture. There is good evidence from the pre thrombolysis era that severe necrosis with infarct expansion predisposes to this complication. It is possible that thrombolysis may also cause cardiac rupture through secondary intramyocardial haemorrhage that disrupts the myocardium by dissecting between the myocyte bundles

Unfortunately these large studies lack detailed information from individual patients and in particular whether thrombolysis was successful in these subjects. This chapter therefore comprises a review of all cases of ventricular septal rupture over a 7 year period (1985-91) when thrombolysis was used in the Royal Infirmary of Edinburgh. By comparing those that did and did not receive thrombolytic therapy it has been possible to assess changes in the presentation of this complication resulting from this therapy.

METHODS

Identification of Cases

All discharge summaries from the Coronary Care Unit were reviewed for patients admitted between January 1985 and December 1991. In addition, a computer search of the discharge diagnoses of all the patients admitted to the Royal Infirmary of Edinburgh between these dates was performed by the Medical Records Department in the Royal Infirmary and the Information and Statistics Division of the Common Services Agency of the Scottish Health Service. Fifty six cases of ventricular septal rupture post infarction were identified. The diagnosis had been confirmed by appropriate investigation (echocardiogram, R heart catheter or left ventricular angiography) in all cases.

Selection of control group

Forty six patients with acute myocardial infarction uncomplicated by ventricular septal rupture, but who had received thrombolytic therapy, were selected to match the two study groups with respect to age, sex, site of infarction and initial ST elevation.

Electrocardiogram Analysis

There is considerable evidence that reperfusion modifies the extent of ST elevation on a surface electrocardiogram (Hogg et al, 1988; Saran et al, 1990; Bren et al, 1987). Serial ST segment changes were therefore analysed in all available electrocardiograms to assess indirectly spontaneous or thrombolytic reperfusion. The extent of ST elevation was measured 0.08s after the J point in the lead with the maximum ST elevation on the initial electrocardiogram. This analysis was not possible in 5 of the 56 patients with septal rupture, because electrocardiograms were inadequate or missing. The mean difference between repeated measures of ST elevation was 0.5mm (coefficient of variation 23%).

Analysis of Coronary Angiograms

Twenty nine of the 56 cases had undergone coronary angiography. All cine films were reviewed by a single blinded observer (ADH) and the extent of significant coronary disease (defined as a stenosis > 50%) was assessed. Perfusion of the infarct related artery was quantified according to the established TIMI criteria (Chesebro et al, 1987). A score of 0 representing no perfusion and 3 indicating normal perfusion. In addition the presence of perfusion by collateral vessels was noted and graded as either poor or good if present.

Statistics

The Mann Whitney U test was used to compare continuous variables and to assess the significance of the delayed increase in ST segment elevation after 24 hours. Differences in the frequencies of baseline characteristics were tested by chi square analysis. Analysis of covariance was employed to assess significance of differences between

the groups in ST elevation 18 hours after the onset of pain (nadir of ST elevation) while correcting for differences in the time of presentation and the extent of initial ST elevation. If several ECGs were available from an individual patient from within the same time interval the data was averaged so that no patient was represented more than once in any time interval. All tests were performed using the Minitab package (release 6.1, PA, USA). A p value less than 0.05 was deemed to be significant.

RESULTS

Baseline Characteristics (Table 5.1)

Forty two patients, who had not received thrombolytic agents, suffered ventricular septal rupture after myocardial infarction and fourteen patients had this complication after thrombolysis. The groups had similar mean age, site of infarction and peak plasma creatine kinase. Their previous medical history was also similar with respect to hypertension and diabetes, but the thrombolysis group had a significantly higher percentage of patients who had previously had clinical ischaemic heart disease. A sizeable proportion of cases had been exposed to non steroidal anti-inflammatory agents (excluding low dose aspirin) or oral steroid prior to septal rupture (overall 36%), although the proportions were not different in the two groups with septal rupture. The control group tended to have fewer patients receiving these agents (24%). The time between onset of chest pain and ventricular septal rupture did not differ significantly between the two study groups. In addition timing of thrombolysis was similar in the thrombolysis group with septal rupture and the control group.

TABLE 5.1

BASELINE CHARACTERISTICS

| | <u>No Thrombolysis</u> n = 42 | <u>Thrombolysis</u> n = 14 | <u>Control</u> n = 46 |
|----------------------------------|----------------------------------|-------------------------------|--------------------------|
| Sex (M:F) | 18:24 | 10:4 | 24:22 |
| Age (yrs) | 69 ± 7 | 71 ± 9 | 65 ± 12 |
| Site (Inferior: Anterior) | 21:21 | 6:8 | 25:20 |
| Peak Creatine Kinase (u/l) | 2210 ± 1801 | 1963 ± 1058 | 1464 ± 1076 |
| Previous IHD | 13 (31%) | 9 (64%)* | 19 (41%) |
| Previous hypertension | 16 (38%) | 3 (21%) | 12 (26%) |
| Previous diabetes | 6 (14%) | 2 (14%) | 3 (7%) |
| Anti-inflammatory Medication | 16 (38%) | 4 (28%) | 11 (24%) |
| Time to Thrombolysis (hr) | | 4.2 ± 3.3 | 4.4 ± 2.2 |
| Time to Septal Rupture (Days) | 5.1 ± 4.1 | 3.6 ± 2.9 | |

Footnote

1. IHD = Ischaemic heart disease (angina or infarction)
2. Anti-inflammatory medication includes non steroidal anti-inflammatory agents and oral steroids given either prior to myocardial infarct and between infarction and septal rupture. (Low dose aspirin excluded)
3. Continuous variables presented as mean ± SD
4. * represents $p < 0.05$.

Coronary Angiography Findings (Table 5.2)

Twenty nine patients were investigated by coronary angiography as an assessment with a view to cardiac surgery. The infarct related artery was occluded in 80% of cases. The distribution of TIMI scores was similar between the two groups and in no patient was perfusion normal. Visible collateral vessels were absent in the majority of cases (80%). In a small number collateral vessels allowed limited filling of the distal part of the occluded vessel and in only one case did retrograde flow result in complete filling of the blocked artery. There was a preponderance of multivessel disease in both groups.

Serial Electrocardiogram Analysis (Figure 21)

ST elevation in electrocardiograms recorded between 0 and 4 hours after the onset of chest pain was similar in the two groups with septal rupture and in the control group. In addition the fall in ST segment elevation with time after the onset of chest pain was similar in the two groups with septal rupture and in both ST elevation tended to increase around the time of rupture. However on formal testing the extent of ST elevation at 72 hours was not significantly greater than that at 24 hours in any group. The control group showed a different response to the other two groups with a rapid fall to a nadir at 18 hours and no tendency to a secondary increase in ST elevation. This difference was shown by analysis of covariance of the ST elevation at 18 hours while correcting for differences in the initial time of presentation and the initial ST elevation (thrombolysis with rupture vs thrombolysis without rupture, $p=0.04$; no thrombolysis with rupture vs thrombolysis without rupture, $p=0.05$).

TABLE 5.2

FINDINGS ON CORONARY ANGIOGRAPHY
(Number of Patients in Each Category)

| | | <u>No Thrombolysis</u> n = 21 | <u>Thrombolysis</u> n = 8 |
|--|---|----------------------------------|------------------------------|
| <u>Infarct artery</u> | | | |
| TIMI | 0 | 18 | 6 |
| | 1 | 2 | 0 |
| | 2 | 1 | 2 |
| | 3 | 0 | 0 |
| <u>Collaterals</u> | | | |
| None | | 16 | 8 |
| Poor filling | | 4 | 0 |
| Good filling | | 1 | 0 |
| <u>No of Vessels > 50% Stenosis</u> | | | |
| | 3 | 10 | 6 |
| | 2 | 9 | 1 |
| | 1 | 4 | 1 |

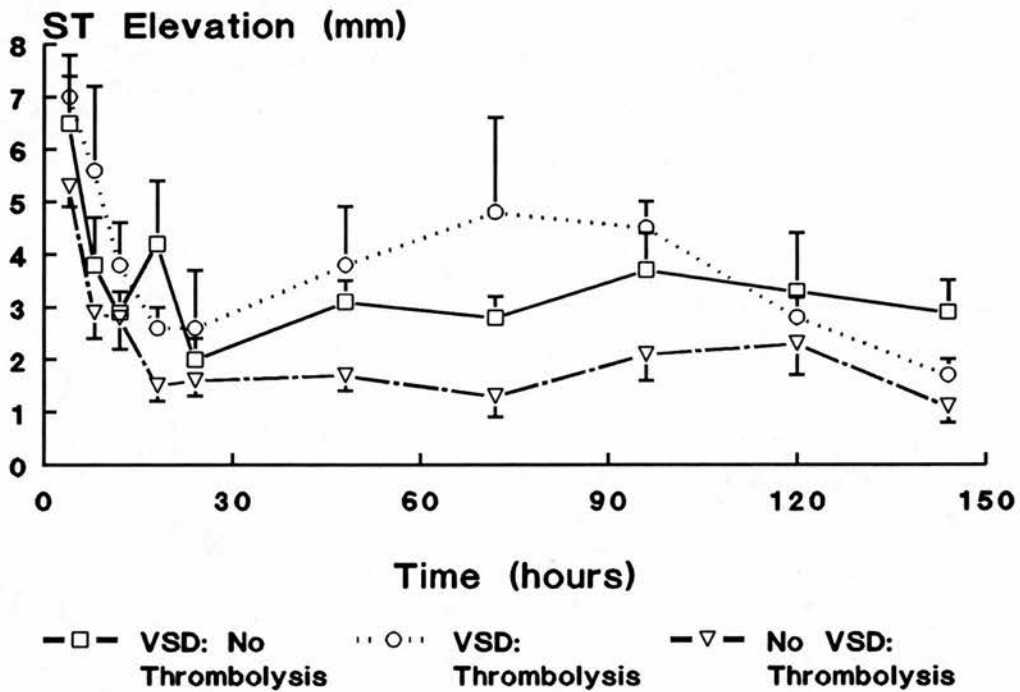


Figure 21 This figure shows the serial changes in ST segment elevation after the onset of chest pain in 97 patients with myocardial infarction. The circles represent data from those who developed ventricular septal rupture (VSR) after thrombolysis ($n=14$), the squares indicate the data from patients with VSR who had not received thrombolysis ($n=37$) and the triangles reflect data from a control group who had received thrombolysis but did not develop VSR ($n=46$). The control patients show a significantly faster reduction in ST elevation down to a lower value and no tendency to further elevation (vs thrombolysis + VSR, $p<0.05$ and vs no thrombolysis + VSR, $p=0.05$). Both groups with VSR tended to show a secondary rise in ST elevation after 24 hours.

After Septal Rupture (Table 5.3)

The clinical diagnosis of septal rupture was confirmed in most patients by echo and doppler cardiography (the remainder by right heart catheterisation). Cardiac catheterisation with coronary angiography was performed in nearly all cases that were considered for surgery. The size of left to right shunt and the pulmonary artery pressure were similar in both groups. The site of septal rupture was evenly distributed along the length of the septum in the non thrombolysis group, but tended towards the apex in the thrombolysis group.

The majority of patients were discussed with the cardiac surgeons (82%), but only 54% underwent surgery.

DISCUSSION

This study indicates that ventricular septal rupture is associated with persisting occlusion of the infarct related coronary artery, whether patients have been treated with thrombolysis or not. This conclusion is supported by the ST segment analysis, as persistent ST elevation is associated with non reperfusion (Hogg et al, 1988; Saran et al, 1990; Bren et al, 1987). Indeed ST elevation decreased faster and remained less in the control group which received thrombolysis, suggesting that a high proportion of these patients had reperfused.

There is substantial evidence that ST segment change is influenced by reperfusion. Hogg et al (1988) found that a fall of greater than 50% in ST elevation 2 hours after thrombolysis was 93% sensitive and 67% specific for reperfusion. Bossaert et al (1991) also demonstrated that ST changes are a useful marker of reperfusion as their data revealed that a

TABLE 5.3

AFTER SEPTAL RUPTURE
(Number of Patients in Each Category)

| | <u>No Thrombolysis</u> n = 42 | <u>Thrombolysis</u> n = 14 |
|--|----------------------------------|-------------------------------|
| <u>Investigations</u> | | |
| Echocardiogram | 34 | 11 |
| Cardiac catheter | 22 | 8 |
| R heart catheter | 26 | 9 |
| <u>Site</u> | | |
| Apex | 12 | 7 |
| Mid ventricle | 12 | 5 |
| Base | 12 | 0 |
| Uncertain | 6 | 2 |
| <u>Shunt</u> (Pulmonary: Systemic ratio) | 2.9 ± 1.6 | 3.5 ± 1.3 |
| <u>Pulmonary artery pressure</u> (mmHg) | | |
| Systolic | 45 ± 12 | 43 ± 8 |
| Diastolic | 20 ± 6 | 19 ± 6 |
| <u>Surgical referral</u> | 35 | 11 |
| <u>Surgery performed</u> | 23 | 7 |

Footnote

Continuous variables mean ± standard deviation

decrease in the sum of ST elevation of more than 50% over the first 2 hours after thrombolysis had a predicative accuracy for reperfusion of 88%. Conversely Saran et al (1990) reported that, when ST elevation does not fall by at least 25% 3 hours after thrombolysis, persistent coronary occlusion is likely (predictive accuracy 86%).

The pathological consequence of the failure of antegrade reperfusion combined with the lack of collateral blood supply is severe and transmural infarction. This was demonstrated in the dog model of infarction by Reimer and Jennings (1979). Further there is now considerable evidence that such patients are those at greatest risk of infarct expansion with dilatation and thinning of the involved segment (Touchstone et al, 1989; De Feyter et al, 1983; Jeremy et al, 1987). The implication is that ventricular septal rupture even after thrombolysis results from pronounced and rapid infarct expansion. It seems likely that the observed secondary rise in ST segments after 24 hours correlates with the process of infarct expansion. One can speculate that persisting or increasing ST elevation is a result of continuing epicardial ischaemia or reflects the side to side slippage of myocytes seen in aneurysm formation.

Previous studies of ventricular rupture have emphasised the importance of occlusive coronary thrombus in its pathogenesis, but all these studies were performed in patients who had not received thrombolysis. London and London (1965) reported 47 cases of cardiac rupture detected amongst 1001 consecutive autopsies post myocardial infarction. Coronary artery occlusion was evident in 76%. In a similar autopsy study in Denmark the incidence of coronary artery occlusion was 71% in

those with cardiac rupture (Rasmussen et al, 1979). Solberg et al (1988) also found that occlusion by coronary thrombosis had occurred in the majority of autopsies after cardiac rupture (74%). Both autopsy and animal studies indicate that persisting occlusion of an artery results in a pale infarct whereas reperfusion causes intramyocardial haemorrhage. In a dog model of infarction 75% of the animals that underwent reperfusion after 6 hours of ischaemia had gross evidence of haemorrhage, in contrast to none of the non reperfused animals (Higginson et al, 1982). In a similar study using anaesthetised dogs Higginson et al (1983) quantified haemorrhage by measuring papillary muscle haemoglobin. Using this technique they established that the duration of ischaemia prior to reperfusion is important. The post reperfusion papillary muscle haemoglobin rose from 14mg/g after 2 hours ischaemia to 28mg/g after 6 hours ischaemia and after 24 hours was 36mg/g. In contrast the nonreperfused animals had a papillary muscle haemoglobin concentration of 8.7mg/g. It seems likely that haemorrhage only occurs after reperfusion in the context of ischaemic damage to the capillary endothelium. It is relevant that streptokinase did not increase the extent of haemorrhage seen after mechanical reperfusion alone in an animal model of infarction (Kloner and Alker, 1984). Haemorrhage was assessed both macroscopically and by measurement of intramyocardial haemoglobin levels. After 3 hours occlusion and 3 hours reperfusion the control group had a myocardial haemoglobin concentration of 26.0/g wet weight compared to the streptokinase treated group which had a concentration of only 12.3 mg/g wet weight. Together with our data this implies that thrombolysis does not cause septal rupture through primary intramyocardial haemorrhage. Indeed it appears to be a failure of thrombolytic therapy

that predisposes to the complication.

Severe haemorrhagic infarction in association with cardiac rupture after thrombolysis has been described. Waller et al (1987) reported on 19 autopsies performed on patients after reperfusion therapy. In these patients myocardial haemorrhage was only seen after thrombolytic therapy and not after reperfusion induced by angioplasty alone. Myocardial rupture was the cause of death of one of the patients in the thrombolysis treated patients. In this case therapy had successfully reopened the occluded artery and apparently was given within 4 hours of the onset of chest pain. A letter in the Lancet reported 2 further cases of patients who had died of cardiac rupture after thrombolysis (Rodgers et al, 1990). At least the first case was in retrospect a patient who presented with subacute cardiac rupture after an infarct 5-10 days earlier. It seems quite possible that one explanation for the increased incidence of cardiac rupture after thrombolysis is in fact inappropriate administration to people with subacute cardiac rupture - a condition which can present in a similar fashion to acute myocardial infarction, with chest pain and ST elevation (O'Rourke, 1973).

In this series we found that 36% of cases had received anti-inflammatory agents prior to ventricular septal rupture compared to 24% of patients in the control group. There is evidence from clinical and animal studies that such treatment may cause an increased tendency to ventricular dilatation by infarct expansion (Roberts et al, 1976; Hammerman et al, 1983a; Boden et al, 1985). If this process is involved in the pathogenesis of cardiac rupture then one would expect such therapy to increase the risk. The interpretation of retrospective

studies such as this are limited as they cannot distinguish between an adverse effect of drug therapy and such therapy being used to treat some aspect of the process of cardiac rupture. This is relevant as non steroid anti-inflammatory agents are used in the treatment of pericardial pain and recurring pericarditic pain is a prominent symptom in those who progress to cardiac rupture (Shapira et al, 1987; Dellborg et al, 1985). However, 25% of the patients (13/52) had been receiving such therapy even prior to their acute myocardial infarction (cf 13% of patients in the control group (6/46), NS). Although this data is not conclusive it certainly would suggest that these drugs should be avoided after infarction if possible.

There is no doubt that septal rupture is a serious complication and it carries a high mortality. Indeed in this study all 26 patients who did not have surgery died. Even with surgical intervention there is a significant mortality and morbidity. Therapy should therefore be mainly directed at prevention. This thesis has already discussed evidence that infarct expansion and ventricular remodelling can be modified by vasodilator therapy with angiotensin converting enzyme inhibitors (Pfeffer et al, 1988; Sharpe et al, 1991) and such therapy might reduce the risk of rupture. In addition the nitrate vasodilators may be beneficial as they have been shown to reduce infarct expansion and to increase collateral blood flow into an ischaemic area (Jugdutt and Warnica, 1988b; Jugdutt et al, 1981). In view of the association of cardiac rupture with persisting arterial occlusion it would be logical that angioplasty might reduce the incidence of cardiac rupture by permitting reperfusion in those patients where thrombolysis was unsuccessful. This has not been addressed in randomised trials. Perhaps the incidence of this

complication can be reduced by such interventions in high risk patients, particularly those with persisting coronary occlusion.

In conclusion, ventricular septal rupture post infarction is associated with persistent occlusion of the infarct related coronary artery. We have found no evidence to suggest that effective reperfusion, and by inference intramyocardial haemorrhage, is important in the pathogenesis of this complication. The incidence of post infarction septal rupture might be decreased by directing therapy towards the prevention of infarct expansion.

CHAPTER 6

CONCLUSION

This thesis illustrates the prime importance of coronary artery reperfusion in the prevention of left ventricular dilatation after myocardial infarction. In the second chapter even the placebo group did not suffer an increase in mean left ventricular dimensions between one and five weeks after infarction. As most of the patients had received thrombolysis, it is likely that ventricular dilatation was inhibited by the high incidence of coronary artery patency. Conversely the data on patients with ventricular septal rupture confirmed that persisting coronary occlusion is detrimental. The process of ventricular rupture is strongly associated with a failure of reperfusion, demonstrated by the angiographic and electrocardiographic data. Ventricular septal rupture appears to occur after severe infarct expansion, a consequence of persisting coronary artery occlusion.

These findings are in agreement with large multicentre trials which reported a benefit from thrombolytic therapy (GISSI-1, 1986; ISIS-2, 1988). Smaller but more detailed studies show that this advantage only occurs in those that reperfuse. Jeremy et al (1987) found that patients with reperfusion did not exhibit ventricular dilatation following myocardial infarction whereas those with persisting occlusion and an absence of collateral blood supply suffered ventricular enlargement over the first 4 weeks.

Another way to reduce the risk of dilatation may be by using vasodilators after acute myocardial infarction. The data from chapter 2 indicated a clinical benefit from early oral vasodilator therapy. Captopril therapy significantly reduced the number of patients either dying or suffering from cardiogenic shock. Such patients are likely to include

those with persisting occlusion of the infarct related artery and those with a large volume of infarcted muscle. However the study failed to show any objective benefit in ventricular size or function in those treated with vasodilator drugs and therefore treatment does not appear to be beneficial to all patients. It is relevant that other trials of vasodilator therapy post infarction have shown a decrease in ventricular enlargement, but only those that selected patients who were at increased risk of dilatation (Sharpe et al, 1991; Pfeffer et al, 1988; Oldroyd et al, 1991). Sharpe et al (1991) selected patients with Q wave infarction. Pfeffer et al (1988) studied patients with anterior infarction who had ejection fractions less than 45%. Oldroyd et al (1991) randomised cases of acute infarction who had not received thrombolysis and had a high Norris score.

In contrast to these reports enalapril therapy did not result in a reduction in the 180 day mortality in the CONSENSUS II study (Swedberg et al, 1992). This may reflect the lack of patient selection prior to inclusion in the study and the possible adverse consequences of early hypotension following intravenous enalaprilat therapy. However in the multicentre SAVE (Survival and Ventricular Enlargement) study captopril therapy proved beneficial (Pfeffer et al, 1992). Subjects with an ejection fraction less than 40% were randomised to treatment with captopril or placebo starting between 3 and 16 days following infarction (mean 11 days). The trial protocol was very careful to exclude patients with evidence of ischaemia (either clinical or on stress testing) and accordingly this selection process may have reduced the number of patients recruited with successful reperfusion. The follow up period was up to 5 years. In this large study captopril reduced mortality, episodes of heart failure and

recurrent infarction. Although the benefit appeared to be independent of ejection fraction, treatment by thrombolysis or Killip class, the study population was still selected to have impaired ventricular function (ejection fraction $<40\%$) and no ischaemia. It is perhaps important that this study only used oral therapy and unlike the CONSENSUS II trial vasodilator therapy was not started early (< 24 hours) when patients are potentially haemodynamically unstable.

Despite the failure of CONSENSUS II to show a benefit, there may still be a role for intravenous vasodilator therapy early after myocardial infarction. Jugdutt and Warnica (1988b) demonstrated that infarct size and expansion was decreased by careful intravenous nitroglycerin therapy avoiding excessive hypotension. Perhaps the proper role for vasodilator therapy will be in patients who have failed to reperfuse. It is still not certain which patients should receive this treatment post infarction and the vasodilator's optimal timing and the best route of administration remain to be defined.

Part of the rationale for the very early introduction of vasodilator therapy was the putative effects of such drugs on infarct size and the acute inflammatory response. The data from chapter 3 indicates that the inflammatory response is not modulated by early oral vasodilator therapy and infarct size is not significantly reduced. The lack of such 'early' effects and the more certain benefit observed when captopril is introduced later after infarction, when haemodynamic stability has been achieved argue for a period of delay prior to initiating vasodilator therapy with an angiotensin converting enzyme inhibitor.

By increasing the proportion of patients with successful reperfusion of the infarct related artery it might be possible to inhibit or even prevent ventricular dilatation after infarction. This might be achieved either pharmacologically by more effective thrombolytic agents or mechanically by transluminal coronary angioplasty. The pharmacological approach is illustrated by the work of Neuhaus et al (1992) who demonstrated that a 'front loaded' rt-PA regime (15mg bolus, 50mg in the first hour and 35mg in the second hour) resulted in an improved patency rate of 84.4% at 90 minutes (cf 70.3% for anistreplase (APSAC) in the same study). Mechanical reperfusion by primary angioplasty is also capable of reopening acute coronary occlusions. O'Keefe et al (1989) reported a series of 500 patients in whom primary angioplasty was attempted and in 94% of cases was successful. Another series found that primary angioplasty attained arterial patency in 91% of patients and was associated with an in-hospital mortality of only 5.6% (Kahn et al, 1990). Trials comparing primary angioplasty to thrombolytic therapy are underway but the results are not yet available in full. Zijlstra et al (1991) reported the preliminary data from one such trial. Of twenty nine patients randomised to streptokinase only 69% had a patent infarct related artery at angiography. In contrast balloon dilatation was successful in all 27 patients in the angioplasty group. Perhaps as a result of vessel patency the pre discharge ejection fraction was significantly greater in the angioplasty group (52% vs 45%, $p < 0.05$). Repeat angiography 2-3 months after the initial admission showed vessel patency was maintained in 94% of the patients treated with angioplasty.

The argument for an aggressive approach to restoration of coronary

artery patency is strengthened by studies of acute revascularisation in cardiogenic shock. Moosvi et al (1992) recently reported a retrospective analysis of 81 patients with cardiogenic shock. Although patients were not randomised, they were selected for inclusion in the analysis using stringent clinical and haemodynamic criteria. Patients revascularised by angioplasty or surgery had a significantly better outcome in comparison to those without revascularisation (50% vs 2% survived to follow up - mean 21 months). However caution should be exercised in interpretation of this data as it was collected retrospectively. Properly conducted randomised studies are still awaited although some people believe such a protocol would not be ethical.

Even if primary angioplasty were shown to be the treatment of choice in acute myocardial infarction, present management might not be changed appreciably. The majority of patients are admitted to hospitals without facilities for acute angioplasty and accordingly thrombolysis is likely to remain the most appropriate therapy for inducing reperfusion. In addition health economics may preclude primary angioplasty even in centres where the technique is feasible.

Prospective trials have shown that routine angioplasty is not advantageous in all patients after thrombolysis. However there is a lack of information on the role of angioplasty in patients that do not reperfuse in response to thrombolytic therapy. The timing of angioplasty in relation to the administration of a thrombolytic agent may be of critical importance. In a paper by Beauchamp et al (1990) patients treated with thrombolysis and angioplasty within 24 hours had an in-hospital mortality of 15.6% in comparison to a mortality of 1.6% if the

angioplasty was delayed more than 24 hours (but less than 4 days). The role of angioplasty may be for selected patients who have contraindications for thrombolytic therapy or appear to have failed to reperfuse. As yet ECG analysis is the most promising method that has been employed to detect such patients non invasively (Hogg et al, 1988; Saran et al, 1990).

Thrombolytic therapy is not without adverse sequelae. The most dramatic are the haemorrhagic complications. Most of the large studies of thrombolytic therapy have shown a small but significant increased risk of bleeding. For instance in the GISSI I study (1986) 0.3% of patients randomised to streptokinase suffered 'major bleeds' in contrast to none in the placebo group. This is also illustrated by the discussion in chapter 5. It seems likely that the inappropriate administration of thrombolytic agents to patients with partial cardiac rupture and chest pain is one reason for the increased incidence of cardiac rupture reported in some large multicentre trials when thrombolytic therapy was administered later after the onset of chest pain. The retrospective review reported in chapter 5 did not demonstrate any relationship between reperfusion by thrombolysis and rupture of the interventricular septum. Rupture probably does not result from intramyocardial haemorrhage that commonly follows reperfusion.

Chapter 4 illustrates another potentially adverse effect of streptokinase. Significant complement and neutrophil activation occurring in patients without reperfusion may increase the extent of necrosis. The observed pattern of complement activation suggests that the direct action of plasmin on the complement factors is the most likely trigger for this

response. There is no evidence to support an antigen-antibody mediated reaction and this may partly explain the lack of correlation between anti-streptokinase antibody titres and successful reperfusion. However further research is required to elucidate the full mechanism and this might in turn lead to therapy to block excessive activation. It is interesting that even an infusion of tissue plasminogen activator is associated with significant anaphylatoxin release (Bennett et al, 1987). It seems unlikely that primary angioplasty will be associated with a similar degree of acute inflammatory activation and this may be an advantage of primary balloon dilatation over thrombolysis.

The link between myocardial necrosis and neutrophil activation is borne out in both chapters 2 and 3 of the thesis. The question of adjuvant therapy to modulate neutrophil or complement function remains theoretical but at least the experimental data is promising. Perhaps the most likely agent to be tried in man is the complement blocking, soluble inhibitory protein (CR1) which reduced infarct size by 44% in a rat model of infarction (Weisman et al, 1990). Careful clinical trials are required to ensure benefit and no tendency to impaired healing and scar formation. Complement activation by thrombolytic agents appears to occur as a result of plasmin generation. Studies are required to clarify whether anaphylotoxin release and the resulting neutrophil activation are in fact clinically harmful. Free radical activity is evident in the later period after myocardial infarction as shown in chapter 3. However this activity does not appear to be related to reperfusion, at least using the free radical markers that were employed in chapter 4. The role of free radical damage in acute myocardial infarction in man remains confusing and this is perhaps a result of difficulty in detecting these reactive

molecules and in proving their involvement in tissue injury in man.

In conclusion, the restoration of coronary artery patency is of primary importance after acute myocardial infarction and at present thrombolytic therapy remains the appropriate treatment for most patients. Published work does suggest a role for angioplasty, but this remains to be defined. Oral vasodilator therapy is beneficial after infarction but perhaps only to selected patients and only after haemodynamic stability is achieved. The acute inflammatory response is activated in myocardial infarction and may be excessively stimulated in some patients treated by thrombolytic therapy with streptokinase. The role of drug therapy to modify such a response in man should be tested, but with caution.

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APPENDIX

PUBLICATION

Hargreaves AD, Kolettis T, Jacob AJ, Flint L, Turnbull LW, Muir AL, Boon NA. Early vasodilator therapy in acute myocardial infarction: appropriate for the majority or the minority? *Br Heart J* 1992; 68: 369-73.

PRESENTATIONS

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